



# Acta OTO-LARYNGOLOGICA

VOL 85 . JANUARY-FEBRUARY 1978 . No 1-2

EDITOR. C-A HAMBERGER STOCKHOLM

## EDITORIAL BOARD

DENMARK O ELBRØND O JEPSEN

H K. KRISTENSEN N RISKÆR H SØRENSEN P STOKSTED

FINLAND J KÄRJÄ O H NEURMAN A PALVA T PALVA

NORWAY J HALL E. STEEN F WINTER

SWEDEN G AŞCHAN B BARR H DIAMANT B DRETTNER C M ENEROTH

H ENGSTRÖM O HALLÉN S INGELSTEDT J WERSÄLL

7

ROBERT HEILIG LIBRARY  
S M ~ N o c i College,  
Jaipur

Acc No \_\_\_\_\_  
Date \_\_\_\_\_  
Dwrg \_\_\_\_\_

DISTRIBUTED BY

THE ALMQVIST & WIKSELL PERIODICAL COMPANY  
STOCKHOLM, SWEDEN

## COLLABORATORS

- Austria* L Horbst, F Krejci, E H Majer, O Novotny, E Schlander, S Unterberger  
*Canada* W J McNally, J A Sullivan  
*Denmark* H C Andersen, J Falbe Hansen, Th Vilstrup  
*Finland* H Björk, B Grahne, U Siirala, E Vaheri  
*France* M Aubry, L G Chevance, G Greiner, P L Mounier-Kuhn, M Portmann  
*Germany* A Herrmann, H G Loebell, A Miehke, R. Mittermaier, H H Naumann, K. H Vosteen, H Wullstein, F Zollner  
*Great Britain* G H Bateman, I S Hall, D F N Harrison, R. D Owen  
*Greece* J Chryssikos, L Papangelou, G E Yannoulis  
*India* J V De Sa, A B N Rao, C Satyanarayana P N Sinha  
*Italy* M Arslan, E Bocca F Brunetti  
*Japan* T Daito, T Fukuda, M Goto, I Kinkae, M Morimoto, J Ono, S Sato  
*Netherlands* L B W Jongkees, W H Struben  
*Norway* P Berdal, H F Fabritius T Leegaard, O Opheim, S Quist Hanssen, O Stromme  
*Sweden* G Dohlman, G Herberts L Holmgren, Hj Koch, G Lidén N Lundgren, C. O Nylén, A Sjöberg  
*Switzerland* F Escher, E Lüscher, A Montandon, C R Pfaltz, L. Ruedi J P Taillens, A Weder  
*USA* L F Boies J E Bordley, T Cody, A C Hilding, D A Hilding P H Höltinger, H P House, G Kelemen, F L Lederer, J R Lindsay, H F Schuknecht, B H Senturia G E Shambaugh, Jr, F A Sooy, W P Work  
*USSR* M Kchodiakov, S Khechinashvili, N A Preobrashensky

### *Conferences and Meetings*

1978, Jan 16-20 The 13th Annual Otologic Surgery Course will be held at the Ear Research Institute in Los Angeles Addr F. H. Linthicum, Jr, M D, Ear Research Institute, 256 South Lake Street, Los Angeles, Calif 90057, USA

1978, Jan 30-Febr 2 ARO Midwinter Research Meeting to be held at the Association for Research in Otolaryngology, Happy Dolphin Inn, St Petersburg Beach, Florida, USA. Pres David J Lim, M D, 4331 University Hospitals Clinic, 456 Clinic Drive, Columbus, Ohio 43210, USA

1978, Febr 5-11 First International Conference of the Politzer Society Inc on Tympanoplasty, Art and Science Review of 25 Years Davos/Switzerland Addr Dr Claus Jansen Winterbeckstr 11, 527 Gummersbach 1, West Germany

1978, Febr 28-March 3 A Seminar on Diagnosis and Management of Acoustic Neuromas and Skull Base Tumors will be held at the Ear Research Institute in Los Angeles Addr William F House, M D, Ear Research Institute, 256 South Lake Street Los Angeles, Calif 90057, USA.

1978, March 8-11 The 2nd International Symposium on Pediatric Otorhinolaryngology will be held in Kansas City Addr B Jazbi M D, Section of Otorhinolaryngology, The Children's Mercy Hospital, 24th at Gillham Road, Kansas City, Miss 64108 USA

1978 April 2-8 Post Graduate Course in Ear Surgery, the Dept of Otolaryngo-

# *Acta* OTO-LARYNGOLOGICA

VOL 85 JANUARY-JUNE 1978 · No 1-6

EDITOR C A HAMBERGER STOCKHOLM

EDITORIAL BOARD

DENMARK O ELBROND O JEPSEN H K KRISTENSEN

N RISKER H SØRENSEN P STOKSTED

FINLAND J KARJA O H MELEMAN A PALVA T PALVA

NORWAY J HALL E STEEN F WINTHER

SWEDEN G ASCHAN B BARR H DIAMANT B DRETTNER C M ENEROTH

H ENGSTRÖM O HALLÉN S INGELSTEDT J WERSALL



## Notes for contributors and subscribers

*Original articles* not published before are accepted in English, French or German. *Manuscripts* should be typewritten, double spaced, on one side only of the paper, followed by a short summary in English and German, and also, if desired, in French and furnished with the author's address. Contributions from Denmark, Finland and Norway should be forwarded to one of the members of the *Editorial Board* of the respective countries, others direct to the Editor, Prof C-A. Hamberger, Karolinska Sjukhuset, Fack, S-104 01 Stockholm 60, Sweden.

Abstract and Summaries should consist of a single paragraph of about 100 (max 150) words.

*References* in the text should be given by author and year, e.g. "as stated by Brown (1953)" or "as earlier reported (Brown, 1953, Brown & Smith, 1956)" and not by figures. In the list of references, the following alphabetical system should be followed, with italics indicated by underlining (title listing *Index Medicus*).

Brown, A. 1953 Obliterative frontal sinusitis *Ann Otol* 62, 377.

— 1954 *Arthritis and Rheumatoid Conditions* John Wiley, New York.

Brown A. & Smith, B. 1956 On the chemistry of the endolymph *Acta Otolaryngol* (Stockh) 46, 408.

*Page proofs* are sent to the author from the printing office and must be returned as soon as possible to Prof C. A. Hamberger, Karolinska Sjukhuset, Fack, S-104 01 Stockholm 60, Sweden. Corrections must be made clearly and no extra matter added.

*Acta Oto-Laryngologica* covers the costs of eight normal printed pages including tables and illustrations. Additional pages and illustrations as well as tables, or coloured illustrations, must be paid for by the author and ought to be avoided as far as possible. No more than one paper by the same author can be accepted for each number.

*Supplements*. Manuscripts exceeding 15 printed pages are published as supplements, the full cost being paid by the author. Supplements are not subject to editorial revision. The references must be listed strictly in accordance with the style of the ordinary articles. In order to help reduce the printing expenses, authors of supplements are invited to suggest suitable full page advertisements for inclusion, subject to the Editor's approval.

*Editorial communications* (incl. advertisements) should be sent to *Acta Oto-Laryngologica*, c/o Prof C-A Hamberger, Karolinska Sjukhuset, Fack, S-104 01 Stockholm 60, Sweden.

*Business communications* (subscriptions, back numbers, supplements and claims) should be addressed to *Acta Oto-Laryngologica*, The Almqvist & Wiksell Periodical Company, Gamla Brogatan 26, S-101 20 Stockholm 1, Sweden.

The subscription rate per regular volume, including supplements published simultaneously, is Sw kr 135 00, payable in advance, post free.

*Back volumes* (supplements not included) will be available for Sw kr 135 00 post free.

*Supplements* containing 100 pages or less cost Sw kr 40 00 per copy, post free. Those in excess of 100 pages cost Sw kr 50 00 per copy, post free.

# CONTENTS

<i>Borg, E.</i> (Stockholm, Sweden) Peripheral Vasoconstriction in the Rat in Response to Sound I Dependence on Stimulus Duration . . . . .	153
<i>Moffat, D. A., Gibson, W. P. R., Ramsden, R. T., Morrison, A. W. and Booth, J. B.</i> (London, Great Britain) Transtympanic Electrocochleography during Glycerol Dehydration . . . . .	158
<i>Manley, G. A.</i> (Montreal, Canada) Cochlear Frequency Sharpening—A New Synthesis . . . . .	167
<i>Legoux, J. P., Teas, D. C., Beagley, H. A. and Remond, M. C.</i> (Paris, France) Relation between the Waveform of the Cochlear Whole Nerve Action Potential and its Intensity Function . . . . .	177
<i>Parving, A. and Bak-Pedersen, K.</i> (Hellerup, Denmark) Clinical Findings and Diagnostic Problems in Sensori Neural Low Frequency Hearing Loss . . . . .	184
<i>Pirodda, E. and Rinaldi Ceroni, A.</i> (Bologna, Italy) Some Experiments on Temporary Threshold Shifts Produced by Short Tones . . . . .	191
<i>Axelsson, A. and Verlez, D.</i> (Göteborg, Sweden) Vascular Histology of the Guinea Pig Cochlea . . . . .	198
<i>Annko, M. and Sarkady, L.</i> (Stockholm, Sweden) Cochlear Pathology Following Exposure to Mercury . . . . .	213
<i>Axelsson, A. and Lindgren, F.</i> (Göteborg, Sweden) Hearing in Pop Musicians . . . . .	225
<i>Tjellström, A., Lundström, J., Albrektsson, T., Bränemark, P.-I. and Hallén, O.</i> (Göteborg, Sweden) A Clinical Pilot Study on Preformed Autologous Ossicles II . . . . .	232
<i>Rosenhall, U., Nylén, O., Lindberg, J. and Kankkunen, A.</i> (Göteborg, Sweden) Auditory Function after Haemophilus Influenzae Meningitis . . . . .	243
<i>Boedts, D. and Kuipers, W.</i> (Antwerpen, Belgium and Nijmegen, The Netherlands) Epithelial Migration on the Tympanic Membrane . . . . .	248
<i>Takahashi, M., Igarashi, M. and Reschke, M. F.</i> (Houston, USA) Directional Conflict between Vestibular and Visual Inputs in the Squirrel Monkey . . . . .	253
<i>Troiani, D., Petrasid, L. and Pallentini, E. A.</i> (Genoa, Italy) Neural Discharge of Medial Geniculate Body Units and Single Semicircular Canal Stimulation . . . . .	262
<i>Teig, E., Dahl, H. A. and Thorkelsen, H.</i> (Oslo, Norway) Actomyosin ATPase Activity of Human Laryngeal Muscles . . . . .	272
<i>Tegner, H.</i> (Lund, Sweden) Quantitation of Human Granulocyte Protease Inhibitors in Non-purulent Bronchial Lavage Fluids . . . . .	282
<i>Ciges, M., Gonzalez, M. and Ceballos, A.</i> (Granada, Spain) Desquamation on Taste Buds . . . . .	290
<i>Ask, P. and Tibbling, L.</i> (Linköping, Sweden) A Simple Device Measuring Differences in Level in the Oesophagus . . . . .	296
<i>Carlöö, B. and Östberg, Y.</i> (Umeå, Sweden) Ultrastructural Observations on the Parotitis Auto-immunica in the NZB/NZW Hybrid Mice . . . . .	298

## Notes for contributors and subscribers

*Original articles* not published before are accepted in English, French or German. *Manuscripts* should be typewritten, double spaced, on one side only of the paper, followed by a short summary in English and German, and also, if desired, in French and furnished with the author's address. Contributions from Denmark, Finland and Norway should be forwarded to one of the members of the *Editorial Board* of the respective countries, others direct to the Editor, Prof C.-A Hamberger, Karolinska Sjukhuset, Fack, S-104 01 Stockholm 60, Sweden.

Abstract and Summaries should consist of a single paragraph of about 100 (max 150) words.

*References* in the text should be given by author and year, e.g. "as stated by Brown (1953)" or "as earlier reported (Brown, 1953, Brown & Smith, 1956)" and not by figures. In the list of references, the following alphabetical system should be followed, with italics indicated by underlining (title listing *Index Medicus*).

Brown, A. 1953 Obliterative frontal sinusitis *Ann Otol* 62, 377.

— 1954 *Arthritis and Rheumatoid Conditions* John Wiley, New York.

Brown, A. & Smith, B. 1956 On the chemistry of the endolymph *Acta Otolaryngol* (Stockh) 46, 408.

*Page proofs* are sent to the author from the printing office and must be returned as soon as possible to Prof C.-A Hamberger, Karolinska Sjukhuset, Fack, S 104 01 Stockholm 60, Sweden. Corrections must be made clearly and no extra matter added. *Acta Oto-Laryngologica* covers the costs of eight normal printed pages including tables and illustrations. Additional pages and illustrations as well as tables, or coloured illustrations, must be paid for by the author and ought to be avoided as far as possible. No more than one paper by the same author can be accepted for each number.

*Supplements*. Manuscripts exceeding 15 printed pages are published as supplements, the full cost being paid by the author. Supplements are not subject to editorial revision. The references must be listed strictly in accordance with the style of the ordinary articles. In order to help reduce the printing expenses, authors of supplements are invited to suggest suitable full page advertisements for inclusion, subject to the Editor's approval.

*Editorial communications* (incl. advertisements) should be sent to *Acta Oto-Laryngologica*, c/o Prof C.-A Hamberger, Karolinska Sjukhuset, Fack, S-104 01 Stockholm 60, Sweden.

*Business communications* (subscriptions, back numbers, supplements and claims) should be addressed to *Acta Oto-Laryngologica*, The Almqvist & Wiksell Periodical Company, Gamla Brogatan 26, S 101 20 Stockholm 1, Sweden.

The subscription rate per regular volume, including supplements published simultaneously, is Sw kr 135 00, payable in advance post free.

Back volumes (supplements not included) will be available for Sw kr 135 00 post free.

Supplements containing 100 pages or less cost Sw kr 40 00 per copy, post free. Those in excess of 100 pages cost Sw kr 50 00 per copy, post free.

# CONTENTS

<i>Palva T Tiesleff I and Savén L</i> (Helsinki Finland) Organ Culture Studies on Human Skin and Cholesteatoma Epithelium	307	<i>Koenig E., Allum J H J and Dichgans J</i> (Tübingen and Freiburg BRD) Visual Vestibular Interaction upon Nystagmus Slow Phase Velocity in Man	397
<i>Aantaa E. and Vrolainen E</i> (Turku, Finland) The Pre- and Postoperative ENG Findings in Clinical Otosclerosis and the Late Hearing Results	313	<i>Stefanelli M Mira E Schmid R and Lombardi R</i> (Pavia Italy) Quantification of Vestibular Compensation in Unilateral Meniere's Disease	411
<i>Arlinger S D., Kylan P and Hellquist H</i> (Lundköping, Sweden) Skull Distortion of Bone Conducted Signals	318	<i>Taguchi K. Iijima M and Suzuki T</i> (Matsumoto Japan) Computer Calculation of Movement of Body's Center of Gravity	420
<i>Gundersen, T and Molander O I</i> (Trondheim, Norway) Hearing Loss Resulting from Perilymph Fistula	324	<i>Wilson, H and Yates M S</i> (Liverpool England) Sympathetic Nerves and Nasal Secretion in the Cat	426
<i>Albert P W Morgan P P and Czuba I</i> (Toronto, Canada) Speech and Pure Tone Audiometry as a Screen for Exaggerated Hearing Loss in Industrial Hearing Claims	328	<i>Eccles R.</i> (Cardiff Wales Great Britain) The Domestic Pig as an Experimental Animal for Studies on the Nasal Cycle	431
<i>Borg E</i> (Stockholm Sweden) Peripheral Vasoconstriction in the Rat in Response to Sound	332	<i>Mygind, N Sørensen H and Pedersen C B</i> (Copenhagen, Denmark) The Nasal Mucosa during Long Term Treatment with Beclomethasone Dipropionate Aerosol	437
<i>Marsh R R Hoffman H S and Stitt C</i> (Bryn Mawr PA USA) Reflex Inhibition Audiometry	336	<i>Hiley C R Wilson, H and Yates M S</i> (Liverpool England) Identification of $\beta$ -Adrenoceptors and Histamine Receptors in the Cat Nasal Vascularature	444
<i>Kawabata I and Nomura Y</i> (Tokyo Japan) Extra Internal Hair Cells	342	<i>Karduck A and Bock W J</i> (Essen BRD) Rhinological Findings Following Transsphenoidal Surgery of the Pituitary Gland	449
<i>Annko M</i> (Stockholm, Sweden) Reversible and Irreversible Changes of the Stria Vascularis	349	<i>Salén, B and Zakrisson J E.</i> (Umeå, Sweden) Electromyogram of the Tensor Tympani Muscle in Man during Swallowing	453
<i>Wicke W., Welleschick B Firsbas W and Schneider H</i> (Wien, Austria) Zur Streptomycinschädigung des Ganglion Spirale	360	<i>Borgesen S and Strømme-Christensen E.</i> (Copenhagen Denmark) The Modern Treatment of Oesophageal Strictures Using the Eder Puestow Dilators	456
<i>de Brey H B and Eggermont J J</i> (Leiden the Netherlands) The Influence of Cochlear Temperature on the Electrical Travelling Wave Pattern in the Guinea Pig Cochlea	363		
<i>Bouchard K R. and Bentler J T</i> (Royal Oak, MI USA) Ultrasonic Irradiation through the Round Window	372		
<i>Igarashi M Takahashi M and Homick J L.</i> (Houston, TX, USA) Optokinetic Afternystagmus and Postrotatory Nystagmus in Squirrel Monkeys			

## Notes for contributors and subscribers

*Original articles* not published before are accepted in English, French or German. *Manuscripts* should be typewritten, double spaced, on one side only of the paper, followed by a short summary in English and German, and also, if desired, in French and furnished with the author's address. Contributions from Denmark, Finland and Norway should be forwarded to one of the members of the *Editorial Board* of the respective countries, others direct to the Editor, Prof C-A Hamberger, Karolinska Sjukhuset, Fack, S 104 01 Stockholm 60, Sweden.

Abstract and Summaries should consist of a single paragraph of about 100 (max. 150) words.

*References* in the text should be given by author and year, e.g. "as stated by Brown (1953)" or "as earlier reported (Brown, 1953, Brown & Smith, 1956)" and not by figures. In the list of references, the following alphabetical system should be followed, with italics indicated by underlining (title listing *Index Medicus*).

Brown, A. 1953. Obliterative frontal sinusitis. *Ann Otol* 62, 377.

— 1954. *Arthritis and Rheumatoid Conditions*. John Wiley, New York.

Brown, A. & Smith, B. 1956. On the chemistry of the endolymph. *Acta Otolaryngol* (Stockh) 46, 408.

*Page proofs* are sent to the author from the printing office and must be returned as soon as possible to Prof C-A Hamberger, Karolinska Sjukhuset, Fack, S 104 01 Stockholm 60, Sweden. Corrections must be made clearly and no extra matter added.

*Acta Oto-Laryngologica* covers the costs of eight normal printed pages including tables and illustrations. Additional pages and illustrations as well as tables or coloured illustrations, must be paid for by the author and ought to be avoided as far as possible. No more than one paper by the same author can be accepted for each number.

*Supplements*. Manuscripts exceeding 15 printed pages are published as supplements, the full cost being paid by the author. Supplements are not subject to editorial revision. The references must be listed strictly in accordance with the style of the ordinary articles. In order to help reduce the printing expenses authors of supplements are invited to suggest suitable full page advertisements for inclusion, subject to the Editor's approval.

*Editorial communications* (incl. advertisements) should be sent to *Acta Oto-Laryngologica*, c/o Prof C-A Hamberger, Karolinska Sjukhuset, Fack, S 104 01 Stockholm 60, Sweden.

*Business communications* (subscriptions, back numbers, supplements and claims) should be addressed to *Acta Oto-Laryngologica*, The Almqvist & Wiksell Periodical Company, Gamla Brogatan 26, S-101 20 Stockholm 1, Sweden.

The subscription rate per regular volume, including supplements published simultaneously, is Sw. kr 135.00 payable in advance, post free.

Back volumes (supplements not included) will be available for Sw. kr 135.00 post free.

Supplements containing 100 pages or less cost Sw. kr 40.00 per copy, post free. Those in excess of 100 pages cost Sw. kr 50.00 per copy, post free.



For more than twenty years, Marianne Coyet has served *Acta Oto Laryngologica* in the capacity of Editorial Secretary, but has now made up her mind to retire, with effect from January 1, 1978

Under Frenckner's editorship, as well as during my own time, Marianne has attended in a most creditable way to the smooth running of *Acta Oto Laryngologica*. She has devoted considerable personal interest to our journal and has kept up the tradition of our founder, Gunnar Holmgren, of endeavouring always to maintain the highest professional standards attainable. In the course of time, Marianne has become great friends with a large number of scientists working within our sphere of interest—something that has facilitated our exceedingly good working relationship with authors and readers.

As the Editor of *Acta*, I feel it my pleasurable duty to convey publicly our acknowledgement of and hearty thanks to a most loyal and invaluable colleague

*Carl-Axel Hamberger*



## VOLUME DISPLACEMENT OF THE TYMPANIC MEMBRANE AT STAPEDIUS REFLEX ACTIVITY IN DIFFERENT POSTURES

### *Studies on Variations in Perilymphatic Pressure*

M Casselbrant, S Ingelstedt and A Ivarsson

*From the ENT Department Malmö General Hospital University of Lund Malmö Sweden*

(Received September 5 1976)

**Abstract** With the microflow method the volume displacement of the tympanic membrane and its direction of movement can be recorded at stapedius reflex contraction. There is an outward or inward movement of the tympanic membrane which is affected by changes in posture. The results indicate that the perilymphatic pressure in man varies with the posture and that these variations can be measured indirectly outside the tympanic membrane. This can only be done, however, on condition that there are no pressure variations across the tympanic membrane. This method opens up new ways of studying possible pressure changes in the inner ear in acute diseases.

The intention of the present authors was to investigate possible variations in the perilymphatic pressure in man and to find out if such variations can be measured outside the tympanic membrane. This investigation seemed to be of particular interest since it enabled us to observe whether the perilymphatic pressure varies with the symptoms of different types of acute sensorineural hearing loss, such as Ménière.

In an experimental investigation on human temporal bone, Densert et al. (1977) showed

that the volume displacement of the tympanic membrane at standardized elicited pull on the stapedius tendon was affected by different perilymphatic pressures. The results seem to indicate that any variations in the perilymphatic pressure in man can be measured by the volume displacement of the tympanic membrane during contraction of the stapedius muscle.

Does the perilymphatic pressure vary with posture? Jonson & Rundcrantz (1969) showed a pressure increase in the internal jugular vein, coinciding with a change from sitting to recumbent position. This increase amounted to about 10 cm H<sub>2</sub>O, identical with the intracranial pressure increase demonstrated by Dawson (1967).

Experimental studies on animals have been performed by Ahlen (1951) and Keith & Allen (1963), who found that pressure in the cerebrospinal fluid is reflected in the perilymph and that an increase in the CSF pressure increased the perilymphatic pressure to the same extent. Beentjes (1972) showed that a change in the CSF pressure affects the perilymphatic as well as the endolymphatic pressure.

We assume that the pressure of the inner ear in man increases to the same extent as the venous and the CSF pressure when the posture is changed from sitting to recumbent. In

These results were reported at Lakarsällskapets Riksstämman Stockholm 1975.

This study was supported by the Swedish Medical Research Council (no B77 17X-04981-01) and the Swedish National Defence Research Institute (proj no 506 H352).



vestigations on humans have been performed by Corso (1962), Miltich (1968) and Macrae (1972), who made comparative determinations of the hearing threshold at changes in posture and were able to show a lowering of the hearing threshold in inverted body position. According to Miltich and Macrae this may be caused by increased intralabyrinthine pressure. Macrae (1972) also studied the change in acoustic impedance with change in posture, and Klockhoff et al (1966) showed similar impedance changes on compression of the neck veins. None of these authors have taken into account the change in middle ear pressure which occurs when the posture is changed or when the neck veins are compressed. This may affect the results.

Andreasson et al (1976) studied the effect on the middle ear mucosa of vein pressure increase, which occurs when the posture is changed from sitting to recumbent. They found that the increased congestion of the middle ear mucosa (about 10  $\mu$ l) in the recumbent position causes an increase in the middle ear pressure amounting to +1 cm H<sub>2</sub>O, when compared with the sitting position with intact tympanic membrane and closed Eustachian tube. With the impedance method, in making measurements with a closed auditory canal, the variations of the congestion in the middle ear, the tension of the tympanic membrane and the skin in the auditory canal may influence the results when the body position is changed.

In an earlier investigation the authors Casselbrant et al (1977) have presented an open microflow method for quantitative recording of the volume displacement of the tympanic membrane upon stapedius reflex contraction. The intention was also to see what middle ear variables influence the stapedius reflex response in a sitting position.

The aims of the present investigation were to find out

1) if possible shifts in the hearing threshold due to changes in posture are caused by changes in the middle ear pressure

2) if the stapedius reflex response is in-

fluenced by changes in posture and the static middle ear pressure,

3) if the change in the perilymphatic pressure at different postures can be measured inside the tympanic membrane.

## METHODS

### *Measuring of the hearing threshold*

A Békésy audiometer (E800 Grason Stadler) was used to determine the hearing threshold by an interrupted fixed frequency tone of 1 kHz during 3 min.

### *Measuring of the middle ear pressure*

An electroacoustic impedance bridge (ZOMED Madsen) was used, by which the middle ear pressure was estimated from a tympanogram at a pressure change in the auditory canal  $\pm 20$  cm H<sub>2</sub>O.

### *Measuring of the stapedius reflex response*

The stapedius reflex contraction was elicited by acoustic stimulation (1 kHz 105 dB re 15  $\mu$ Pa) and recorded in the contralateral ear with an open microflow method by which the movement direction of the tympanic membrane and its volume displacement can be recorded quantitatively (For details, see Casselbrant et al, 1977).

### *Changes in posture*

The investigations were made with the subject sitting in a heart chair which could be adjusted so that the upper half of the body was in the position planes of 85° (sitting), 30°, 20°, 10° and 0° (recumbent). S<sub>1</sub>-S<sub>4</sub> means sitting position and R<sub>1</sub>-R<sub>2</sub> recumbent. The values are the mean values from 10 recordings made during 3 min after the subject had equilibrated the middle ear properly during 5 min.

## MATERIAL

Ten subjects belonging to tubal function group Ib the same as were used by Casselbrant et al

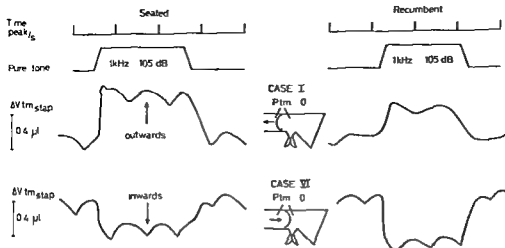


Fig 1 Recording in two cases of outward (case I) or inward (case VI) movements of the tympanic membrane at stapedius reflex contraction in seated and recumbent

positions.  $P_{tm}$  pressure difference across the tympanic membrane

(1977). The subjects were also able to equilibrate the middle ear to atmospheric pressure in a sitting as well as in a recumbent body position.

## PERFORMANCE AND RESULTS

### *Determination of hearing threshold in sitting and recumbent positions*

It was checked at the start that the subjects in the sitting position had atmospheric pressure in the middle ear and that they either did or did not equilibrate the middle ear pressure during investigations in the recumbent position.

There was no statistical difference ( $p > 0.05$ ) between the hearing thresholds in the sitting and the recumbent position, whether the subjects had equilibrated or not.

**Comments.** During the investigation of the hearing threshold at different middle ear pressures a change of 2.2 dB ( $p < 0.001$ ) was followed by a relative overpressure in the middle ear of about 2 cm H<sub>2</sub>O as compared with the atmospheric pressure in the middle ear (Casselbrant et al., 1977).

### *Determination of middle ear pressure in sitting and recumbent positions*

The middle ear pressure was examined in the sitting and the recumbent position with and without equilibration of the middle ear. The middle ear pressure increased, on average, by +1 cm H<sub>2</sub>O (S.D. = 0.34, S.E. = 0.1) in the recumbent position, as compared with the sitting position when the subjects did not equilibrate.

## VOLUME DISPLACEMENT OF TYMPANIC MEMBRANE AT STAPEDIUS REFLEX CONTRACTION

### *(a) In sitting and recumbent position*

An acoustically elicited stapedius reflex contraction causes either an outward or an inward movement of the tympanic membrane in a sitting position (Casselbrant et al., 1977). When the posture is changed from the sitting to the recumbent, the stapedius reflex response pattern measured as the volume displacement of the tympanic membrane will be affected when a pure tone stimulus (1 kHz, 105 dB) is produced in the contralateral ear. (Fig. 1)

Table I The initial volume displacement of the tympanic membrane,  $\Delta V_{tm, \text{stap}}$ , in the sitting position ( $S_1$ - $S_3$ ) and in the recumbent position ( $R_1$ - $R_3$ ) at pure tone stimulus (1 kHz 105 dB) ISO 1964) produced in the contralateral ear

Middle ear pressure =  $P_{tm}$

The initial volume displacement is the mean value from 10 recordings in each body position

+ = outward movement - = inward movement of the tympanic membrane

Case	Left (probe ear)					Right (probe ear)				
	$S_1$	$R_1$	$S_2$	$R_2$	$S_3$	$S_1$	$R_1$	$S_2$	$R_2$	$S_3$
I	+0.50	+0.38	+0.53	+0.42	+0.52	+0.37	+0.28	+0.36	+0.30	+0.40
II	+0.40	+0.33	+0.39	+0.30	+0.38	+0.44	+0.46	+0.46	+0.42	+0.46
III	+0.40	+0.34	+0.37	+0.32	+0.37	No rec	No rec	No rec	No rec	No rec
IV	+0.10	-0.08	+0.12	-0.06	+0.10	+0.09	-0.04	+0.05	-0.04	+0.04
V	+0.06	-0.11	+0.06	-0.09	+0.05	+0.20	-0.08	+0.21	-0.09	+0.23
VI	-0.32	-0.53	-0.29	-0.49	-0.36	-0.38	-0.54	-0.32	-0.48	-0.35
VII	+0.10	0	+0.10	+0.03	+0.13	-0.14	-0.21	-0.15	-0.23	-0.18
VIII	+0.11	0	+0.11	0	+0.13	+0.04	-0.06	+0.04	-0.04	+0.03
IX	+0.54	+0.54	+0.54	+0.54	+0.53	+0.30	+0.14	+0.30	+0.17	+0.27
X	-0.06	-0.20	-0.07	-0.20	-0.07	-0.09	-0.16	-0.12	-0.17	-0.11

Table I gives the initial volume displacement of the tympanic membrane at stapedius reflex contraction (cf initial volume displacement, Casselbrant et al, 1977). The values are the mean values from 10 recordings in each body position. The subjects had equilibrated the middle ear pressure to atmospheric after each change of posture.

Subjects with great outward movement of the tympanic membrane in the sitting position show a smaller outward movement in the recumbent position (cases I, II, III and IV bilateral, see Table I). In all cases except two (cases II right and IX left), there are statistically significant changes ( $p < 0.001$ ).

Subjects whose tympanic membranes move inwards in the sitting position get a stronger inward movement in the recumbent position (cases VI-X bilateral and VII right, see Table

I). The change is statistically significant for these ears ( $p < 0.001$ ).

Subjects with a slight outward movement in the sitting position show a change in the movement direction of the tympanic membrane to an inward movement in the recumbent position (cases IV, V bilateral and VIII right, see Table I).

#### (b) Gradual change in posture

The stapedius reflex response in different postures has also been studied in 2 subjects (cases V and VI) with the upper half of the body in the positions of 85° (sitting), 30°, 20°, 10° and 0° (recumbent), respectively. Immediately after each change in posture the subjects equilibrated the middle ear pressure to ambient pressure. Table II shows the difference

Table II The initial volume displacement of the tympanic membrane at stapedius reflex contraction  $\Delta V_{tm, \text{stap}}$  at gradual change in posture

The values are the mean values from 10 recordings in each posture

+ = outward movement - = inward movement at 1 kHz 105 dB rel ISO 1964

Case	Ear	S	20°	S <sub>1</sub>	10°	S <sub>2</sub>	0°	S <sub>3</sub>	30°
V	Left	+0.05	0.03	+0.04	0.07	+0.05	-0.11	+0.06	+0.01
	Right	+0.23	0.10	+0.18	+0.04	+0.20	-0.07	+0.16	+0.12
VI	Left	-0.33	0.35	0.32	-0.41	-0.33	-0.48	-0.32	No rec
	Right	-0.36	0.38	-0.35	-0.40	0.37	-0.52	-0.38	No rec

Table III Double examinations of the initial volume displacement of the tympanic membrane, ( $\Delta V_{tm\text{stap}}$ ), in the sitting ( $S_1$ - $S_3$ ) and the recumbent ( $R_1$ - $R_3$ ) position at pure tone stimulus (1 kHz, 105 dB rel ISO 1964) produced in the contralateral ear

The middle ear pressure =  $P_{\text{aem}}$   
The initial volume displacement = the mean value from III recordings in each posture

Case	Examination	Left					Right				
		$S_1$	$R_1$	$S_2$	$R_2$	$S_3$	$S_1$	$R_1$	$S_2$	$R_2$	$S_3$
I	1st	+0.50	+0.38	+0.53	+0.42	+0.52	+0.37	+0.28	+0.36	+0.30	+0.40
	2nd	+0.50	+0.31	+0.51	+0.42	+0.51	+0.36	+0.30	+0.39	+0.31	+0.38
V	1st	+0.06	-0.11	+0.06	-0.09	+0.05	+0.20	-0.08	+0.21	-0.09	+0.23
	2nd	+0.10	-0.07	+0.08	-0.08	+0.07	+0.15	-0.06	+0.14	-0.04	+0.13
VI	1st	-0.32	-0.53	-0.29	-0.49	-0.36	-0.38	-0.54	-0.32	-0.48	-0.35
	2nd	-0.34	-0.50	-0.33	-0.48	-0.32	-0.41	-0.45	-0.35	-0.46	-0.36

ence in the "response" amplitude recorded at 0°, 10°, 20° and 30° body angle in relation to response amplitude in the sitting position ( $S_1$ ,  $S_2$ ,  $S_3$  and  $S_4$ ). The essential change in the stapedius reflex response occurs at 10° body angle

#### (c) Time factor after change in posture

In case V the influence of the time factor on the stapedius reflex response was studied after a change from the sitting to the recumbent position. The essential change of the stapedius reflex response occurred immediately

#### (d) Reproducibility of stapedius reflex response

As an illustration of the reproducibility of the volume displacement of the tympanic membrane at stapedius reflex contraction in different postures, Table III presents results of re examinations of 3 subjects (5 ears). In these 3 subjects we found good agreement between the recorded volume displacements of the tympanic membrane during the different examinations. These took place at intervals of several months

### INFLUENCE OF STAPEDIUS REFLEX CONTRACTION ON MOVEMENT DIRECTION OF TYMPANIC MEMBRANE

A comparison of the results of the volume displacement of the tympanic membrane and

its movement direction at stapedius reflex contraction in different postures (Table I) and at different middle ear pressures (Casselbrant et al., 1977) shows that the material can be divided into four groups

Group I an outward movement of the tympanic membrane in the sitting and the recumbent position and at different middle ear pres-

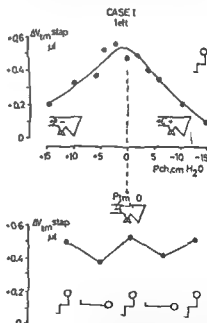


Fig. 2 Volume displacement of the tympanic membrane at stapedius reflex contraction ( $\Delta V_{tm\text{stap}}$ ) at different middle ear pressures (upper curve) produced in a pressure chamber ( $P_{\text{aem}}$ ) in the sitting position and in the sitting-recumbent position (lower curve) with atmospheric pressure in the middle ear 1 e group I

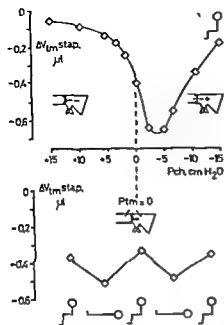
CASE VI  
right

Fig. 3 Group II

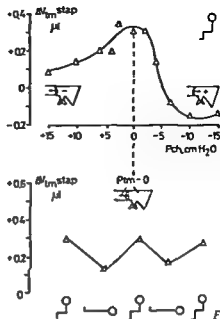
CASE IX  
right

Fig. 4 Group III

tures. Example of recording in Fig. 2. The material consisted of 5 ears (3 subjects).

**Group II** an inward movement of the tympanic membrane in the sitting and recumbent position and at the different middle ear pressures. Example of recording in Fig. 3. The material consisted of 5 ears (3 subjects).

**Group III** an outward movement of the tympanic membrane in the sitting and the recumbent position. There is a change of movement direction at relative overpressure, i.e. when the tympanic membrane is outside its neutral position (Fig. 4). The material consisted of 4 ears (3 subjects).

**Group IV** an outward movement of the tympanic membrane in the sitting position. When the tympanic membrane is outside its neutral position (relative overpressure in the middle ear) and in the recumbent position there is a change of the movement direction to an inward movement (Fig. 5). The material consisted of 5 ears (3 subjects).

## DISCUSSION

A comparison between the sitting and the recumbent position shows a change in sta-

pedius reflex response, recorded as the volume displacement of the tympanic membrane at identical middle ear pressures. In some cases there is also a change in its movement direction at an acoustically elicited stapedius reflex contraction (Fig. 5). The stapedius reflex response patterns, recorded outside the tympanic membrane, are identical with those found by Densert et al. (1977) in results from

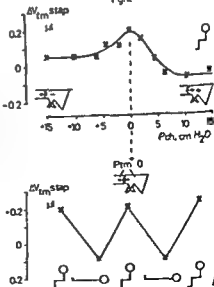
CASE X  
right

Fig. 5 Group IV

experimental investigations on human temporal bones. These authors showed that the volume displacement of the tympanic membrane at artificial stapedius reflex contraction was affected by different perilymphatic pressures. At increased perilymphatic pressure they recorded a gradual decrease in the volume displacement of the tympanic membrane when the movement direction of the tympanic membrane was outward. They recorded the reflex response upon momentarily loading (1.3 g) the tendon of the M. stapedius. They also found that one temporal bone showed a change in the movement direction of the tympanic membrane from outward to inward movement at an increased perilymphatic pressure.

The agreement between the present results as regards the volume displacement of the tympanic membrane at stapedius reflex contraction and the model experiments on human temporal bones by Densert et al. (1977) indicates that the perilymphatic pressure increases with a change in body position from sitting to recumbent. This may be assumed to be due to an increase in the pressure in the internal jugular vein (Jonson & Rundcrantz 1969) and of the CSF pressure (Dawson 1967). Another parallel with the internal jugular vein pressure appears from the finding of Jonson & Rundcrantz (1969) that the essential pressure increase occurs at the change in posture from 20° to 0° (horizontal plane). This agrees with the results of our investigation showing the change in the volume displacement at stapedius reflex contraction following change in posture (Table II). The greatest change in the volume displacement of the tympanic membrane at stapedius reflex contraction occurs near the horizontal plane (10°-horizontal plane). It is thus improbable that the difference in the volume displacement of the tympanic membrane at stapedius reflex contraction is caused by the changed gravitational force exerted on the ossicular chain upon change of posture.

When it comes to determining possible

variations in the perilymphatic pressure by recording the volume displacement of the tympanic membrane at stapedius reflex contraction there is a risk of error. As has been shown (Casselbrant et al. 1977) the stapedius reflex response and hearing threshold are affected by the middle ear pressure. At a change from the sitting to the recumbent position there is a pressure increase of on an average 1 cm H<sub>2</sub>O due to the increased congestion in the middle ear mucosa if the middle ear is not equilibrated after the change in posture.

It should be observed that as little as 1 cm H<sub>2</sub>O relative overpressure in the middle ear can markedly affect the stapedius reflex response (Fig. 3). A relative overpressure of 1 cm H<sub>2</sub>O in the middle ear does not affect the hearing threshold. This is in agreement with the results of Mütch (1968) and Macrae (1972).

The increase in hearing threshold and acoustic impedance which these authors found during investigations in the inverted position have mainly been ascribed to the increase in intralabyrinthine pressure. However, in the inverted position our investigations show a middle ear pressure increase of 3-5 cm H<sub>2</sub>O which must be taken into account.

There is another possible source of error to be avoided in impedance measuring, namely the congestion in the tympanic membrane and the skin of the auditory canal at changes in posture. The results of impedance measurements are dependent on the volume change of the auditory canal. With our open microflow method this error can be avoided.

Our results together with the results from the model experiment by Densert et al. (1977) on human temporal bones show that it is possible to measure variations in the perilymphatic pressure on the outside of the tympanic membrane—provided that the middle ear pressure can be controlled. This is further confirmed by the reproducibility of the stapedius reflex response between different examination occasions (Table III).

An attempt should be made to explain why

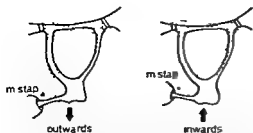


Fig 6 An explanation of the different movement directions of the tympanic membrane at stapedius reflex contraction

the tympanic membrane at stapedius reflex contraction has different opposite movement directions. The reason should be sought in the anatomical relations between the stapes and the stapedius tendon. At stapedius reflex contraction according to Bel et al (1976), there is a pedal movement of the stapedius foot plate in the oval window. If the stapedius tendon when leaving the eminentia pyramidalis is located outside the stapedius head, a contraction of the stapedius muscle will cause an outward movement of the tympanic membrane (Fig 6 outwards). On the other hand, if the tendon leaves the eminentia inside the head of the stapes, it will cause an inward movement (Fig 6 inwards).

Why is the volume displacement of the tympanic membrane at stapedius reflex contraction affected by the middle ear pressure or body position and why do some ears show a reversal in movement direction? A decrease of the pressure in the auditory canal or an increase in the perilymphatic pressure results in pushing the stapedius foot plate out of the oval window (Densert et al 1977). In this way the relation between the head of the stapes and the stapedius tendon attachment is changed, which in its turn results in a change in the volume displacement of the tympanic membrane. In some ears even the direction of the movement is reversed.

After assessing the middle ear pressure according to a technique described by Elnor et al (1971) it is possible by changing the surrounding pressure, to bring the tympanic

membrane into its neutral position and to make measurements of the variations in the perilymphatic pressure in subjects not belonging to tubal function group Ib.

## ACKNOWLEDGEMENT

The authors are grateful to Ass. Prof. Henry Andersen, Department of Audiology, Karolinska Sjukhuset, Stockholm, for his valuable discussion and constructive suggestions.

## ZUSAMMENFASSUNG

Durch Messungen der Volumenschwankung im Gehörgang können mit einer Mikroströmungsmethode die Lageveränderungen sowie die Bewegungsrichtung des Trommelfells bei stapediusreflexbedingter Kontraktion registriert werden. Es wurden Trommelfellbewegungen nach innen oder nach außen registriert, bei denen die Körperstellung von Einfluß war. Die Resultate zeigen, daß sich der perilymphatische Druck beim Menschen mit der Körperstellung ändert und daß diese Druckänderungen indirekt außerhalb des Trommelfells gemessen werden können. Dies ist allerdings nur unter der Voraussetzung möglich, daß keine Druckänderungen über dem Trommelfell erfolgen. Die Methode eröffnet neue Möglichkeiten für das Studium etwaiger Druckänderungen im Innenohr bei akuten Erkrankungen.

## REFERENCES

- Ahlen G 1951 On the connection between cerebrospinal and intralabyrinthine pressure and pressure variations in the inner ear. *Acta Otolaryngol* (Stockh) 35: 251.
- Beentjes B 1972 The cochlear aqueduct and the pressure of the cerebrospinal and endolabyrinthine fluids. *Acta Otolaryngol* (Stockh) 73: 112.
- Bel J, Causse J, Michaux P, Cezard R, Canu Y & Tapons J 1976 Mechanical explanations of the On-Off effect (Disphasic impedance change) in otospongiosis. *Audiology* 15: 128.
- Casselbrant M, Ingelstedt S & Ivarsson A 1974 Volume displacement of the tympanic membrane in a sitting position as a function of middle ear muscle activity. *Acta Soc Med Scand* 84: 386.
- 1977 Volume displacement of the tympanic membrane in a sitting position as a function of middle ear muscle activity. A quantitative microflow method. *Acta Otolaryngol* (Stockh) 84: 402.
- Corso J F 1967 Bodily position and auditory threshold. *Perceptual and Motor Skills* 14: 449.
- Dawson H 1967 *Physiology of the cerebrosplinal system*. J and A Churchill Ltd, London.
- Densert O, Ivarsson A & Pedersen K 1977 Pressure variations in the middle ear during stapedius reflex contraction. *Acta Otolaryngol* (Stockh) 84: 402.

- lymphatic pressure influence on the volume displacement of the tympanic membrane. A quantitative study on temporal bones. *Acta Otolaryngol* (Stockh) **84**, 220
- er, A., Ingelstedt, S. & Ivarsson, A. 1971 Indirect determinations of the middle ear pressure. *Acta Otolaryngol* (Stockh) **72**, 255
- nson, H. & Rundcrantz, H. 1969 Posture and pressure within the internal jugular vein. *Acta Otolaryngol* (Stockh) **68**, 271
- ith, J. & Allen, G. 1963 Comparison of the perilymphatic and cerebrospinal fluid pressure. *Arch Otolaryngol* (Stockh) **77**, 581
- ockhoff, I., Änggård, G. & Änggård, L. 1966 Record ing of cranio-labyrinthine pressure transmission in man by acoustic impedance method. *Acta Otolaryngol* (Stockh) **61**, 316
- Macrae, J. H. 1972 Effects of body position on the auditory system. *J Speech Hear Res* **15**, 330
- Miltch, A. J. 1968 Human auditory threshold shifts following changes between upright, supine and inverted bodily position. *J Aud Res* **8**, 367
- Margaretha Casselbrant M.D.  
ENT department  
Malmö General Hospital  
S-21401 Malmö  
Sweden



## THE EFFECTS OF THE VACUUM ON VASCULAR PERMEABILITY OF THE MIDDLE EAR

F Hiraide<sup>1</sup> and H Ernksson<sup>2</sup>

From the <sup>1</sup>Department of Otolaryngology University of Tokyo Tokyo Japan and the <sup>2</sup>Department of Otolaryngology University of Minnesota Medical School Minneapolis MI USA

(Received February 25 1977)

**Abstract** Following local application of various degrees of negative pressure to the middle ear the authors observed changes in vascular permeability in guinea pigs using Majno's vascular labelling technique. Increased permeability and effusions were seen in all experimented middle ears. Any pressure below -5 mm of mercury induced middle ear effusions. Accordingly a decrease in pressure developed in the middle ear cavity may cause transudation of serum drawn from the submucosal vessels resulting in fluids in the middle ear of aero-otitis. In such a circumstance mast cells which are easily influenced by atmospheric pressure may be involved in increasing vascular permeability.

It is a well known fact that aero-otitis media or aural barotrauma is a disease which occurs as a result of sudden change in atmospheric pressure during flying at high altitudes, working in a caisson or diving in deep waters. The phenomenal growth of commercial air transport or exploratory travels in space makes these problems of special interest and importance to the general medical profession.

The common symptoms of aero otitis media are characterized by pain, tinnitus, injection and invagination of the tympanic membrane, hearing loss, effusion or hemorrhage in the middle ear cavity.

Several experimental studies have appeared in the literature to clarify the pathogenesis of the middle ear effusion in such circumstances (Aschan, 1948; Flisberg et al 1963; Paparella et al, 1970).

In our present study, we have attempted to observe the vascular change of permeability in the middle ears of guinea pigs using Majno's vascular labelling technique (Majno et al 1961) after locally applying various degrees of negative pressure to the middle ear cavity.

### MATERIAL AND METHOD

Seventeen healthy guinea pigs weighing 220-400 mg were used in this study. The animals were anesthetized with Urethane. A sub-mandibular approach was applied to reach the tympanic bulla. After exposure of the tympanic bulla, a tiny opening was made with a 2 mm round cutting bur. The tip of a fine polyethylene tube was inserted. It was sealed by packing gauzes containing physiological saline. Subsequently, a colloidal suspension of carbon particles (200 Å) was injected into the external jugular vein (0.1 ml/100 g of body weight). Groups of experimental animals were subjected to varying degrees of negative pressure, for a fixed duration of time. The negative pressure was supplied by a regular suction machine with which the negative pressure could easily be controlled to the desired value.

The animals were sacrificed about 30-45 minutes after the injection of carbon black when almost all carbon has been cleared from

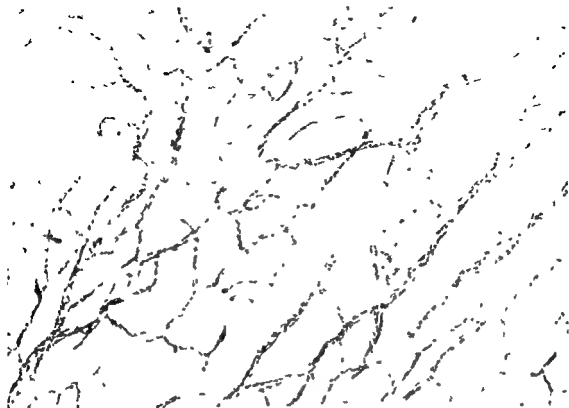


Fig. 1. Surface preparation of guinea pig middle ear mucosa after applying negative pressure of  $-5$  mmHg. A highly developed arboreal pattern of blackened vessels

indicates that considerable leakage of carbon has occurred ( $\times 100$ ).

culating blood. The middle ear mucosa was removed and prepared as surface specimens for observation under the dissection microscope. They were placed in 10% formalin solution for 24 hours, dehydrated in gradient filtered ethanol and then placed in xylene to make them clear. Several ears were used for conventional histopathological study with ematoxylin and eosin stain. Light microscopic observation was carried out in these specimens.

## RESULTS

A negative pressure of  $-5$  mmHg was applied to the middle ears of 4 guinea pigs for 5 minutes. The cleared middle ear mucosa from 1 treated guinea pig showed a slightly developed arboreal pattern of blackened vessels,

indicating that considerable leakage of carbon had taken place (Fig. 1). However, labelling was limited to capillaries, venules and small veins. No tendency of extending to adjacent tissues was noted. Arterioles and artery showed no carbon leakage throughout their walls. A considerable amount of amber colored, clear, and low viscous middle ear fluid was obtained from all treated ears. Their cytology showed that these effusions contained essentially no cells nor cellular materials, although occasional red blood cells were found. Histologically, middle ear mucoperiosteum removed at this stage was slightly edematous, without any indication of hemorrhage. Small blood vessels labelled with carbon were clearly distinguished. No pathologic findings were observed in control ears.

The middle ears of 4 guinea pigs were exposed for 5 minutes at the negative pressure of



2 Sectioned specimen of guinea pig middle ear mucosa after applying negative pressure of  $-10$  mmHg

Vascular labelling is seen in the walls of small blood vessels and subepithelial edema is noted ( $\times 200$ )

$-10$  mmHg Carbon labelling of small vessels was more extensive than that seen at previous stages, but was similar in distribution. In addition, local hemorrhages were sometimes found in the submucosa of treated middle ears. The areas corresponding to hemorrhages appeared

tained from 2 out of 4 guinea pigs. Cytology revealed that 2 remaining ears also contained a considerable amount of red blood cells. However, these fluids had no tendency to coagulate. Histologically, the mucoperiosteums appeared to be edematous in most cases (Fig. 2). Occasional submucosal hemorrhages were detected.

For a period of 5 minutes the middle ears of 3 guinea pigs were subjected to a negative

pressure of  $-20$  mmHg of mercury. Carbon labelling revealed more increased vascular permeability in small blood vessels.

Bloody middle ear fluids were obtained from all treated ears. The mucoperiosteums were edematous and succulent in all cases. Local hemorrhages were noted frequently at this stage.

Three guinea pigs were subjected, for 10 minutes, to a negative pressure of  $-30$  mmHg in the middle ears.

A remarkable increase in vascular permeability was noted in the small vessels of vacuum treated middle ears. Heavy labelling was visible throughout their walls. The extensive vascular blackening was also noted in the middle ears of the other 3 experimental animals. Bloody fluids were obtained in all cases. Cytology revealed the

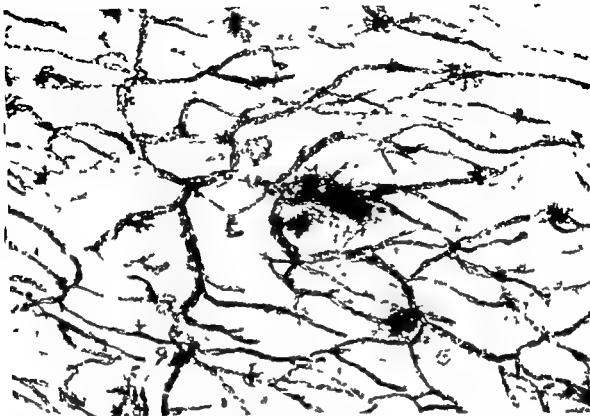


Fig 3 Surface preparation of middle ear mucosa after applying negative pressure of 50 mmHg. Vascular labelling by carbon particles is clearly observed. The ex-

travascular blackening is present in the adjacent tissues of small blood vessels ( $\times 200$ )

most were red blood cells. Histologically the mucoperiosteums showed interstitial edema but no emigration of leukocytes was noted.

A negative pressure of  $-50$  mmHg was applied to the middle ears of 3 guinea pigs for 5 minutes.

The appearance of the cleared middle ear mucoperiosteums was similar to that of the animals exposed to negative pressure of  $-30$  mmHg. The degree of blackening in small vessels, however, was outstandingly extensive compared with any of the previous cases. In addition to capillaries, venules and veins, and also arterioles may also be involved. The carbon deposits were confined to the walls of the affected vessels. Furthermore, various degrees of extravascular blackening were always present in adjacent tissues of small blood vessels of experimental middle ear mucosa (Figs

3-4). It was probably caused by leakage of carbon particles through the endothelial gaps or ruptured blood vessels. The fluids obtained from treated middle ear cavity were bloody. Some of them tended to coagulate, indicating that they might have consisted partly of blood.

Histologically the mucoperiosteums showed edema in all cases. The labelling of small vessels with carbon was clearly observed in sectioned specimens. Hemorrhages were noted to a varying extent in the vacuum-treated middle ear mucosa. No perforation of the tympanic membrane was noted in any of the experimentally treated animals.

## DISCUSSION

Leaking blood vessels can be marked *in vivo* by intravenously injecting a colloidal suspen-



Fig. 2. Sectioned specimen of guinea pig middle ear mucosa after applying negative pressure of  $-10$  mmHg.

Vascular labelling is seen in the walls of small blood vessels and subepithelial edema is noted ( $\times 200$ ).

$-10$  mmHg Carbon labelling of small vessels was more extensive than that seen at previous stages, but was similar in distribution. In addition, local hemorrhages were sometimes found in the submucosa of treated middle ears. The areas corresponding to hemorrhages appeared black due to the extension of carbon into adjacent tissues. Bloody middle ear fluids were obtained from 2 out of 4 guinea pigs. Cytology revealed that 2 remaining ears also contained a considerable amount of red blood cells. However, these fluids had no tendency to coagulate. Histologically the mucopenosteums appeared to be edematous in most cases (Fig. 2). Occasional submucosal hemorrhages were detected.

For a period of 5 minutes, the middle ears of 3 guinea pigs were subjected to a negative

pressure of  $-20$  mmHg of mercury. Carbon labelling revealed more increased vascular permeability in small blood vessels.

Bloody middle ear fluids were obtained from all treated ears. The mucopenosteums were edematous and succulent in all cases. Local hemorrhages were noted frequently at this stage.

Three guinea pigs were subjected, for 4 minutes, to a negative pressure of  $-30$  mmHg in the middle ears.

A remarkable increase in vascular permeability was noted in the small vessels of vacuum treated middle ears. Heavy labelling was visible throughout their walls. The extensive vascular blackening was also noted in the experimented animals. Bloody fluids were obtained in all cases. Cytology revealed the

min. It was a typical transudate—a thin, ear yellow fluid.

It is likely that negative pressure applied to the middle ear creates an abnormally low hydrostatic pressure causing small blood vessels (venules and many of capillaries) to produce an exaggerated amount of ultrafiltrate or transudate. Additionally, mast cells, which are distributed in the normal middle ear mucosa, may participate in increasing vascular permeability (Hiraike & Paparella, 1972). The mast cells are stimulated by changing atmospheric pressure and then histamine or another mediator is released, causing vasodilation and leakage of regional vessels.

As demonstrated in an animal experiment by Lamkin et al. (1975), positive as well as negative pressure influences the middle ear mucosa with occasional transudation of serous fluid and hemorrhage. This suggests that positive pressure can stimulate mast cells in the middle ear mucosa to release chemical mediators such as histamine, serotonin, and others, which subsequently cause the vasodilation and production of middle ear fluid.

Persistent negative pressure leads first to tympanic edema and then transudation. Transudation of blood serum from capillaries and venules into the tympanic cavity may take place in tubal occlusion due to absorption of oxygen as soon as the pressure state in the middle ear cavity falls below that of the plasma protein osmotic pressure. Several investigators have measured the under pressure of the diseased human middle ear cavity (Dischoeck, 1941; Perlman, 1943; Thomsen, 1958). They found negative pressures varying down to a maximum of  $-60$  cm  $H_2O$  and presence of middle ear fluids.

Holmgren (1940) also measured negative pressure equivalent to  $5-7$  cm  $H_2O$  in the tympanic cavity in dogs, of which the Eustachian tube was operatively occluded.

Matsumura (1955) analysed the middle ear gases of cats after cauterization of the pharyngeal orifice of Eustachian tubes. He found direct correlation between the degree of tubal

obstruction and depression of oxygen levels.

Elner (1976) has calculated the rate of gas absorption from the human middle ear. He has determined the mean value to be  $33 \mu l/l$  hour or  $0.7-1.1$  ml/24 hours under normal conditions. On the basis of recordings of the pressure drop in the ear in cases of perforated tympanic membrane, Riu et al. (1966) have calculated the absorbed volumes and found approximately the same value ( $0.8$  ml/24 hours).

The ear is a nearly rigid chamber which can decrease its volume very little only by inward movement of the tympanic membrane. When the Eustachian tube is closed the middle ear cavity becomes a closed chamber. Gas absorption occurs gradually and the underpressure grows in the closed middle ear cavity. When this reaches a certain limit, transudation occurs due to the increase in vascular permeability.

After experimentally obstructing the Eustachian tubes, an obvious increase in vascular permeability invariably occurred in the submucosa of squirrel monkeys and guinea pigs (Paparella et al., 1970; Hiraike & Paparella, 1972).

Accordingly, transudate from capillaries is the main component of the fluid in the early stage of serous otitis media.

We are of the opinion that the blood vessels undoubtedly participate in the production of the middle ear fluids in ear-otitis media and presumably in serous otitis media.

## ACKNOWLEDGEMENTS

The authors are deeply grateful to Dr M. M. Paparella for his valuable suggestions and Miss Haruko Miyazaki for her help in preparing this manuscript.

## ZUSAMMENFASSUNG

Durch lokale Applikation des negativen Druckes von vielerlei Graden am Mittelohr eines Meerschweinchens beobachteten wir die Veränderungen der vaskulären Permeabilität nach der Majnoschen vaskulären etikettieren der Technik. Im experimentellen Mittelohr waren zunehmende Permeabilität und Effusionen gefunden. Alle

Drucke unter  $-5$  mmHg veranlassen die Mittelohreffusion. Also die Absteigerung des Druckes in der Mittelohrhöhle mag die Transudation des Serums von subepithelialen Gefäßen verursachen, welche aerootische Flüssigkeit ergeben hat. Im solchen Zustande mögen die Mastzellen vom Druck der Atmosphäre leicht beeinflusst in der zunehmenden vaskulären Permeabilität enthalten werden.

## REFERENCES

- Aschan □ 1948 Acro-otitis media and aerosinusitis *Acta Otolaryngol* (Stockh) Suppl 69 1
- Dishoeck H A E 1941 Negative pressure and loss of hearing in tubal catarrhal *Acta Otolaryngol* (Stockh) 29 303
- Elner Å 1976 Normal gas exchange in the human middle ear *Ann Otol Rhinol Laryngol* Suppl 25 161
- Flisberg K, Ingelstedt S & Ortegren U 1963 On middle ear pressure *Acta Otolaryngol* (Stockh) Suppl 182 43
- Hiraide F & Paparella M M 1972 Vascular changes in middle ear effusions *Arch Otolaryngol* 96 45
- Holmgren L 1940 Experimental tubal occlusion *Acta Otolaryngol* (Stockh) 28 587
- Lamkin R, Axelsson A, McPherson D & Miller J 1975 Experimental aural barotrauma: Electrophysiological and morphological findings *Acta Otolaryngol* (Stockh) Suppl 335
- Majno G, Palade E & Schoeff I 1961 Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree. A topographic study *J Biophys Biochem Cytol* 11 607
- Matsumura H 1955 Studies on the composition of air in the tympanic cavity *Arch Otolaryngol* 61 270
- Paparella M M, Hiraide F, Juhn S K & Kaneko 1970 Cellular events involved in middle ear fluid production *Ann Otol Rhinol Laryngol* 79 766
- Perlman H B 1943 Quantitative tubal function *Arch Otolaryngol* 38 453
- Riu R, Flottes L, Bouche J et al 1966 *Physiologie la Trompe d'Eustache*. Librairie Arnette Paris
- Thomsen K A 1958 Investigations on the tubal function and measurement of the middle ear pressure in pressure chamber *Acta Otolaryngol* (Stockh) Suppl 14 269

F Hiraide MD  
Dept of Otolaryngology  
University of Tokyo  
Hongo Bunkyo-ku  
Tokyo  
Japan

## DIE LACTATDEHYDROGENASE (LDH) DES INNENOHRES NACH LARMBELASTUNG

M Ishida

*Aus der Hals Nasen Ohren Klinik, Das Zentrum für Erwachsenen Krankheiten  
(The Center for Adult Diseases) Osaka, Japan*

(Eingegangen am 8. November, 1976)

**Abstrakt** Diese Abhandlung beschreibt die histologischen und biochemischen Studien der LDH Vorgänge des Cortiorgans, der Stria Vascularis und der Perilymphe unter akustischer Überreizung. Die Tiere wurden über einen Zeitraum von 10 min bis 72 Std mit einem „Rosa Rauschen“ einer Frequenz von 50 Hz bis 10 KHz in einem diffusen Schallfeld beschallt. Der Lärmpegel betrug in jedem Fall 115 dB. Nach Dauerlärmeinwirkung von einer Stunde konnte eine eindeutige Abschwächung der Färbung in den inneren u. äusseren Haarzellen sowie an den dazugehörigen Nervendingungen festgestellt werden. Wenn die Schallbelastung jedoch aus 15 Stunden ausgedehnt wurde, zeigte sich Erhöhung der LDH Aktivität. Eine biochemische Untersuchung folgte der oben beschriebenen histochemischen Arbeit. Die LDH Aktivität des Cortiorgans und der Stria Vascularis der ersten vier Windungen wurde mit der sogenannten mikrochemischen Technik gemessen.

Diese Abhandlung beschreibt die histologischen und biochemischen Studien der LDH Vorgänge des Cortiorgans, der Stria Vascularis und der Perilymphe unter akustischer Überreizung.

Seit der bekannten Arbeit von Wittmaack (1907) gibt es viele histopathologische Untersuchungen des Innenohres unter Lärmbelastung. Neue Aspekte in der Beurteilung der Stria Vascularis und des Cortiorgans bei Lärmschädigung bringt die Betrachtung biochemischer Vorgänge. Es liegen nur wenige Ergebnisse histochemischer Untersuchungen der LDH vor. Fermentlokalisationen in der Cochlea bei Lärmschädigung wurden von Vosteen (1961 und 1964), Koide (1964), Ger-

hardt (1964) und Vinnikov & Titova (1963) vorgenommen.

Vosteen (1961) bemerkte als Erster einen graduellen Abfall der SDH nach Lärmbelastung, beginnend zuerst in den Nervenendingungen, dann in den ganzen äusseren Haarzellen. Ähnliche Befunde bekamen Vinnikov & Titova (1963) beim Meerschweinchen und Kaninchen. Vosteen interpretierte diese Veränderungen als Ausschöpfung des aeroben Metabolismus. Er vertrat die Ansicht, dass neben dem aeroben glykolytischen Metabolismus ein anaerober Weg existieren musste, der als Notfallreserve bei kurzer Übereinstimmung oder kurzzeitigem Sauerstoffmangel zur Verfügung stehe. Eine Reihe wichtiger Arbeiten auf die quantitative biochemische Bestimmung im Bereich des Innenohres gerichtet. Rauch (1964), Rauch & Plester (1965), Schindler et al. (1965), Schindler & Schnieder (1966), Silverstein & Schuknecht (1966), Lotz & Kuhl (1968), Matschinsky & Thalmann (1967), Thalmann & Matsehskey (1970). Quantitative Angaben über die Pyridinnucleotide in der Stria Vascularis bringt Matschinsky & Thalmann (1967). Von Imura (1967) liegen Ergebnisse über die ATPase in der Stria Vascularis und dem Ligamentum spirale vor.

Um einen Begriff von der Grösse des energieliefernden Stoffwechsels zu bekommen, wurde die LDH in den Stria Vascularis der



einzelnen vier Windungen und dem Cortiorgan der Meerschweinchencochlea untersucht

METHODIK

Es wurden 47 bunte gut horende Meer-schweinchen mit einem Gewicht von 240-360 g verwandt. Die Tiere wurden über einen Zeitraum von 10 Min bis 72 Std mit einem „Rosa Rauschen“ einer Frequenz von 50 kHz bis 10 kHz in einem diffusen Schallfeld beschallt. Der Lärmpegel betrug in jedem Fall 115 dB. Bei allen Tieren wurden direkt nach Beschallungsende in einer Nembutalnarkose durch retroaurikulares Vorgehen die Cochlea freigelegt und je zwei  $\mu$ l Perilymphe durch das Runde Fenster entnommen. Unmittelbar nach der Perilymphentnahme erfolgte die Dekapitation der Tiere und Entnahme beider Cochleae. Die Cochleae wurden unter kalter physiologischer NaCl-Lösung eröffnet und die Stria Vascularis sowie die Cortiorgane einschliesslich der unmittelbar angrenzenden knochenfreien Strukturen der einzelnen vier Windungen separat zur anschliessenden spektrophotometrischen Bestimmung entnommen. Die Proben wurden nach ihrer Entnahme in glasernen Mikroröhrchen in kalter physiologischer NaCl-Lösung eröffnet, homogenisiert und nach Zentrifugieren der klare Überstand für die folgende Bestimmung des Gesamteiwisses und der LDH-Aktivität abpipettiert. Das Gesamteiwiss wurde nach der Folin-Methode bestimmt. Zur spektrophotometrischen Messung diente ein Zeiss-spektral-photometer PMQ II.

Die Kontrolluntersuchung wurden an 14 weiteren Tieren ohne Schallbelastung ermittelt. Anschliessend entfielen zwei Tiere zur morphologischen Untersuchung auf jeden Beschallungsversuch. Die Schnecken wurden wie oben beschrieben von retroaurikular aufgesucht und supravital nach Fixieren des Staples mit kaltem Karnovsky'schen Fixationsgemisch (1965) von Runde Fenster aus perfundiert. Nach ihrer Entnahme und Nachfixation in dem gleichen Gemisch erfolgte die histologische Aufarbeitung in der Technik nach

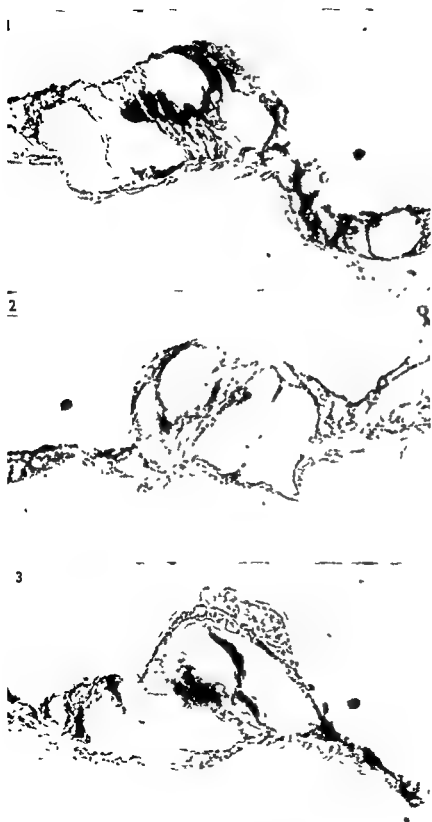
Barker. Das Eindringen des Inkubationsgemisches durch regelmässige Schaukelbewegungen bei 37°C erleichtert. Schliesslich wurden die mit 10% EDTA entkalkten Präparate in Paraplast eingebettet und die angefertigten Schnitte unter einem Zeiss-Forschungsmikroskop beurteilt.

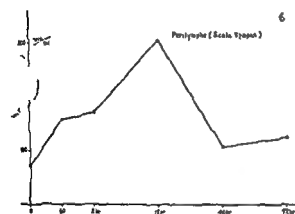
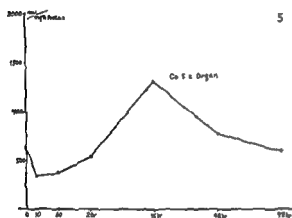
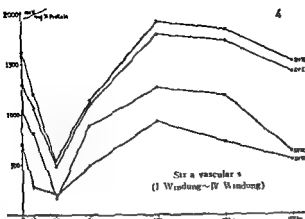
ERGEBNISSE

Als histochemische Ergebnisse bei den normalen Tieren ohne Schallbelastung kam LDH im grossen Umfang in den Haarzellen wobei kein Unterschied in der Aktivität ausser und innerer Haarzellen erkennbar war. Allerdings lagen die Formosanablagerungen in den inneren Haarzellen relativ gleichmässig verteilt im gesamten Cytoplasma. Dagegen wurden etwas kraftigere Ablagerungen vom Farbstoff an der Zone des Nervenendigunges am unteren Zellpol der äusseren Haarzellen (Abb. 1). Nach Dauerlärmeinwirkung von einer Stunde konnte eine eindeutige Abschwächung der Färbung in den inneren und äusseren Haarzellen sowie an den dazugehörigen Nervenendigungen festgestellt werden (Abb. 2). Wenn die Schallbelastung jedoch auf 15 Stunden ausgedehnt wurde, zeigte sich Erhöhung der LDH-Aktivität (Abb. 3).

Eine biochemische Untersuchung folgte der oben beschriebenen histochemischen Arbeit. Die LDH-Aktivität des Cortiorgans und der Stria Vascularis der einzelnen vier Windungen wurde mit der sogenannten mikrobiologischen Technik gemessen. Bei normalen Meerschweinchen zeigten biochemisch das Cortiorgan und Stria Vascularis keinen Aktivitätsunterschied der LDH-erkennbaren

Aus den Kurvenverläufen, wobei auf der Ordinate mU/mg Eiweiss und der Abszisse der Zeitverlauf aufgetragen sind, ist zu entnehmen, dass in den ersten Stunden die LDH-Aktivität der Stria Vascularis der einzelnen vier Windungen abnimmt, nach diesem Zeitraum jedoch sich eine steigende Tendenz zeigt. Nach 15 Stunden kommt es wieder zu einem massigen Aktivitätsabfall. Der Kurvenverlauf für die einzelnen Windungen erscheint fast





tel, wobei die Werte aus der 2ten Windung den höchsten Kurvenverlauf ergeben, gefolgt von der ersten, dritten und vierten (Abb. 4).

Die Kurve des Cortiorganes verläuft ähnlich wie bei der Stria Vascularis. Am Cortiorgan finden wir auch innerhalb der ersten 10 Minuten einen starken Abfall der LDH-Aktivität, gefolgt von einem Anstieg der Aktivität, der nach Beschallung von 15 Stunden sein

Maximum erreicht. Danach zeigt an die LDH einer massigen Abfall der Aktivität (Abb. 4).

Weiter wurde die LDH-Aktivität in der Perilymphe nach Beschallung untersucht. Nach Schallbelastung von 15 Stunden fand sich ein Maximumwert der LDH, 5 mal so hoch wie Kontrollwert. Bis zu 15 Stunden nach der Beschallung zeigte sich eine steil aufsteigende Linie ohne Abfall in der ersten Stunde. Danach weist die starke Abfall wieder auf. Nach mehr als 48 Stunden Beschallung zeigt die Aktivitätskurve einen annähernd ebenen Verlauf, der bei Kontrollwerte entsprechend (Abb. 6).

## DISKUSSION

Der Glucoseabbau im Innenohr erfolgt oxydativ über den Krebszyklus (Citronensäurezyklus), nachdem zunächst glycolytisch Brenztraubensäure gebildet worden ist. Brenztraubensäure kann zu Milchsäure hydriert werden, so dass als Endprodukt des glycolytischen Glucoseabbaues im Innenohr das Lactat angesehen werden kann. Dieser Lactatanteil trägt, bezogen auf die Gesamtmenge der aufgenommenen Glucose 7-8%. Nach den Untersuchungen von Barkus werden zwei Drittel der im Gehirn zur Oxydation zur Verfügung stehenden Glucose zunächst in Aminosäuren überführt, während das restliche Drittel direkt und vollständig zu  $\text{CO}_2$  veratmet wird. Der Gewinn bei Glycolysis beträgt zwar nur 2 ATP pro mol Glucose, aber viel mehr Energie, mag die Umwandlung in Milchsäure nicht liefern. Ausserdem besitzen wir noch ein verhältnismässig energiereiches Substrat - Milchsäure - zum weiteren Abbau, wo Sauerstoff verfügbar ist. Lactat bewirkt immer wie ein Vasodilatator und damit ein Aufrechterhalten der Durchblutungsgrosse. Fehlt doch der Sauerstoff im Wirbeltierorganismus bei der anaeroben Glykolyse des Muskels wird Pyruvat zu Lactat reduziert.

In Analogie zu den Befunden Therman (1963) an der Netzhaut schloss Vosteen eine Abhängigkeit von der unterschiedlichen

Art der Energiegewinnung durch überwiegend anaerobe (LDH) und aerobe Fermentaustattung (SDH). Als Fazit dieser Untersuchungen resultiert eine Gleichung (Schatzle, 1971): gute Durchblutung = gute Sauerstoffversorgung = überwiegend aerober Stoffwechsel (Basale Schneckenwindung, innere Haarzellen) = geringerer Glycogengehalt und geringe oder fehlende Ausstattung mit anaerobtatigen Fermenten.

Schlechtere Durchblutung = schlechtere Sauerstoffversorgung = anaerobtatig (äußere Schneckenwindungen, Glykogengehalt) = gute Ausstattung mit anaerobtatigen Fermenten.

Einen weiteren Beweis, dass aerobe und anaerobe Prozesse bei der Lärmbelastung eine Rolle spielen, lieferten Hayata & Harada (1967). Sie untersuchten Aktivität des SDH und Phosphorylase, das erste Enzym als Parameter des aeroben und das letztere als Parameter des anaeroben Metabolismus. Nach Beschallung mit 100 dB für 3 Stunden konnten Conti & Borgo (1964) eine diffuse Abnahme der Zytochromoxydase im Cortiorgan unabhängig von den benutzten Frequenzen nachweisen. Nach mehrtägiger Beschallung mit einem Dauerton liess sich eine Abnahme der SDH-Aktivität in der Meerschweinchenkochlea beobachten (Vosteen, 1958, 1960a, 1961). Eine zunehmende Beschallungsdauer mit 2000 Hz und 70–85 dB bewirkte eine zunehmende Ausbreitung, des betroffenen Bezirks der zweiten Windung. Im Zentrum des Ansprechgebietes kamen auch histologische Schädigungen vor. Die Abnahme der Fermentaktivität erfolgte zunächst in den Nervenendigungen, dann in der ganzen äußeren Haarzelle. Die inneren Haarzellen bleiben unberührt. Eine Erholung nach Dauerbeschallung trat innerhalb der Nachbeobachtungszeit von 5–6 Wochen nicht ein. Bei Beschallung mit Kurztönen über 2–4 Tage fanden sich nur ausserordentlich geringe Veränderungen. Die Schallschädigung des Cortiorgans soll somit nicht Folge einer mechanischen Zerstörung von

Haarzellen, sondern die letzte Stufe einer Stoffwechselerkrankung der Phonorezeptoren sein. Nach Beschallung beschreiben auch Vinnikow & Titova (1963) eine Abnahme von SDH in den äußeren Haarzellen und Spiralganglionzellen von Meerschweinchen und Kanarienvögeln.

Viele Autoren untersuchten die Sauerstoffabnahme in den endolymphatischen Flüssigkeiten nach Schallbelastung (Misrahy & Hildreth 1958, Koide 1960). Misrahy machte zwei verschiedene Effekte für diesen Sauerstoffmangel verantwortlich. Ein Anwachsen des Sauerstoffverbrauches durch metabolische Haarzellprozesse und die Verminderung der O<sub>2</sub>-Diffusion von der Stria Vascularis in die Endolymph unter Lärmbelastung sprach weitestgehend für diese Idee.

Yamashita (1973) hat schon die LDH und SDH Aktivität am einzelnen Cortiorgan unter verschiedenen Sauerstoffspannungen gemessen und festgestellt, dass über 60 mM der aerobische Atmungsstoffwechsel nicht wirkt, wogegen das anaerobische Glycolyse starke Wirkung zeigt.

Eine Änderung der Aktivität eines Enzyms in einer Körperflüssigkeit oder in einem Organ verschiedene Ursachen haben:

- 1 Die Grundsustanz, an die das Enzym gebunden ist, geht zu Grunde und das Enzym wird mit dem Blut oder der Lymphe ausgeschwemmt.
- 2 Die Permeabilität der Zellmembran ändert sich. Um die Membranpotentiale und Konzentrationsunterschiede aufrecht zu erhalten, verbraucht die Zelle laufend freie Energie.
- 3 Durch eine Änderung des Zellstoffwechsels kommt es zu einer vermehrten Produktion von Enzymen.

Die Änderung der LDH Aktivität nach Beschallung konnte auch durch die obengenannten verschiedenen Prozesse verursacht werden. Der LDH Abfall nach einstündiger Beschallungsdauer, der eine Zunahme der aeroben Glykolyse ausgewiesen haben konnte, ist

deutlich signifikant in der 1 und 2 Windung gegenüber den Ausgangswerten (Kontrollwerten), während diese Signifikant für die 3 und 4 Windung nicht so deutlich ist. Werden die Tiere länger als 60 Minuten beschallt, so kommt es zur Zunahme der LDH-Aktivität, die einem aussteigenden anaeroben Stoffwechsel andeuten könnte.

## ZUSAMMENFASSUNG

Die Schallwirkung auf die LDH Aktivität des Innenohres war Gegenstand dieser Arbeit. An Meerschweinchen wurde der schädigende Einfluss von Lärm auf die LDH-Aktivität der Stria Vascularis, des Cortiorgans und der Perilymphe mit Hilfe biochemischer Methoden untersucht, die eine quantitative Auswertung der Befunde erlauben.

In der Stria Vascularis nimmt die Gewebeaktivität der LDH in den ersten Stunden ab, nach dieser Zeit zeigt sie eine steigende Tendenz. Nach 15 Stunden kommt es jedoch zu einem massigen Aktivitätsabfall. Der Kurvenverlauf für die einzelnen Windungen erscheint parallel, wobei die Ergebnisse aus der 2. Windung den höchsten Kurvenverlauf zeigen, dann kommt in dieser Reihenfolge, die erste, dritte und vierte.

Im Cortiorgan finden wir innerhalb der ersten 10 Minuten nach Beschallung einen sehr starken Abfall der LDH Aktivität, die nach 15 Stunden Beschallung ihr Maximum erreicht.

Im Bereich von 15 bis 48 Stunden Beschallung kommt es parallel zu den Befunden in der Perilymphe zu einem sehr starken Abfall der Aktivität. Nach mehr als 48 Stunden Beschallung finden wir nur noch eine sehr massige Aktivitätsabnahme im Cortiorgan. Bei der Untersuchung der Perilymphe bis zu 15 Stunden Beschallungsdauer sieht man eine steil aufsteigende Linie, die danach wieder einen starken Abfall aufweist. Nach mehr als 48 Stunden Beschallung verläuft die Aktivitätskurve fast flach, die dem Kontrollwert entspricht.

## SUMMARY

This paper deals with histochemical and biochemical studies on LDH activity in the organ of Corti, the stria vascularis and the perilymphatic fluid in the inner ear under acoustic overstimulation.

Forty seven normal guinea pigs with good Preyer reflex were used. These animals were continuously exposed to white noise of 115 dB for periods ranging from 10 minutes to 72 hours. After acoustic overstimulation the inner ears were exposed and perfused with LDH incubation solution through the scala tympani to the scala vestibuli. The animals were decapitated and the inner ears were fixed with Karnovsky's solution and embedded with Paraplast. LDH staining of the Corti organ and stria vascularis showed formation of formosan. When exposed for 60 minutes almost all the formosan was found to have disappeared. However, when exposed for 15 hours the LDH activity was found to have increased again.

A biochemical study followed the above described histochemical study. The LDH activity in the Corti organ and every turn of the stria vascularis was measured by the so-called microbiological technique. The animals were decapitated and the inner ears were placed in physiological saline at about 4°C. The Corti organ and stria vascularis of each turn were obtained separately by the surface preparation and homogenized in fine glass tubes. The homogenate obtained was centrifuged and the supernatant fluid was assayed for LDH activity and protein content. In normal guinea pigs the organ of Corti and all parts of the stria vascularis proved to have almost the same level of activity.

Of the four turns of the stria vascularis the second turn was shown to have the highest activity and to be followed by the first, third and fourth in decreasing order.

When exposed for 60 minutes the activity decreased markedly in all the turns of the stria vascularis and constituted the lowest levels. When exposed for 15 hours the activity attained the highest levels. After 72 hours of exposure however the activity decreased again and returned to the normal levels.

The curve for the Corti organ followed the same pattern as in the stria vascularis. In the case of perilymph the activity peaked at 15 hours (five times the normal level) and then decreased gradually to normal.

## REFERENCES

- Conti A & Borgo M 1964 Behaviour of cytochrome oxidase activity in the cochlea of the guinea pig following acoustic stimulation. *Acta otolaryngol* 59: 38-41.
- Gerhardt H J 1964 Zur Lokalisation der Glucose-6-phosphat und der Gluconat-6-phosphat-dehydrogenase in der Meerschweinischnecke. *Arch Otorinolaryngol* 184: 52.
- Hayata T & Harada Y 1967 Histochemical studies of succinic dehydrogenase and phosphorylase in the cochlea of guinea pigs exposed to white noise. *Actaologica Fukuoka* 13 Suppl. 1: 136.

- Imura T 1967 ATPase in Stria Vascularis *Laryngoscope* 77 141
- Karnovsky M J 1965 A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy *J Cell Biol* 27 137
- Koide Y 1960 Some aspect of the biochemistry of acoustic trauma *Ann Otol Rhinol Laryngol* 69 661
- Koide Y R 1964 Distribution of some oxidizing enzymes in the cochlea *Acta Otolaryngol* (Stockh) 58 344
- Lotz E & Kuhl K D 1968 Die Lactatdehydrogenase des Innenohres *Arch Klin Exp Ohren Nasen Kehlkopfheilkd* 192 237
- Matschinsky F M & Thalmann R 1967 Quantitative histochemistry of microscopic structure of the cochlea *Ann Otol Rhinol Laryngol* 76 639
- Misrahy G A & Hildreth H III 1958 Endolymphatic oxygen tension in the cochlea of the guinea pig *J Acoust Soc Am* 30 247
- Rauch S 1964 Biochemische Alarmsymptome Otogener Labyrinthreizungen *Arch Ohren Nasen Kehlkopfheilkd* 183 453
- Rauch S & Plester 1965 Untersuchung der Perilymph nach stapedeotomierter Otosklerosen *Med Hyg* 23 984
- Schindler K & Schnieder E A 1966 Perilymph in patients with otosclerosis *Ann Otol Rhinol Laryngol* 74 373
- Schindler K, Schnieder E A & Wullstein H L 1965 Vergleichende Bestimmung einiger Elektrolyte und organischer Substanzen in der Perilymphe otosklerosekranker Patienten *Acta Otolaryngol* (Stockh) 59 309
- Schatzle W 1971 *Histochemie des Innenohres* Urban & Schwarzenberg München Berlin Wien
- Silverstein H 1966 Biochemical studies of the inner ear fluids in the cat Preliminary report *Ann Otol Rhinol Laryngol* 75 48
- Silverstein H & Schuknecht H F 1966 Biochemical studies of inner ear fluid in man *Ann Otol Rhinol Laryngol* 84 395
- Thalmann R & Matschinsky F M 1970 Quantitative study of selected enzymes involved in energy metabolism of the cochlea duct *Ann Otol Rhinol Laryngol* 79 12
- Thiemann H 1963 *Elektronenoptische Untersuchungen über das Glykogen in Zellstoffwechsel* Fischer Stuttgart
- Vinnikov J A & Titova L III 1963 Cytophysiology and cytochemistry of the organ of Corti A cytochemical theory of hearing *Int Rev Cytol* 14 157
- Witmaack K 1907 Über Schädigung des Gehörs durch Schalleinwirkung Ein experimentelle Studie *Z Ohrenheilkd* 51 37
- Vorstein K H 1958 II Erschöpfung der Phonorezeptoren nach funktioneller Belastung Experimentelle histochemische Untersuchung zur Frage des Schalltransformation im Innenohr *Arch Ohren Nasen Kehlkopfheilkd* 172 489
- 1960a Die Energieproduktion in Cortischen Organ *Acta Otolaryngol* (Stockh) Suppl 163 54
- 1961 Neue Aspekte zur Biologie und Pathologie des Innenohres *Arch Ohren Nasen Kehlkopfheilkd* 178 1
- 1964 Elektronenmikroskopische Untersuchungen über die Verteilung von Glycogen in Ductus Cochlearis beim Meerschweinchen *Pract Otorhinolaryngol* (Basel) 26 400
- Yamashita T 1973 Histochemical studies on the effects of oxygen deprivation in the hair cells of Corti organ *Arch Klin Exp Ohren Nasen Kehlkopfheilkd* 204 797

M Ishida M D  
Dept of Otolaryngology  
The Center for Adult Diseases Osaka  
Ichome Nakamichi-cho  
Higashinari ku  
Osaka  
Japan

## THE SIZE OF THE MIDDLE EAR AND THE MASTOID AIR CELL *System measured by an acoustic method*

O I Molvær, F M Vallersnes and M Kringlebotn

*From the Department of Otolaryngology the Roentgen diagnostic Department  
and the Acoustic Laboratory ELAB NTH University of Trondheim Trondheim Norway*

(Received July 20 1976)

**Abstract** The total volume for the middle ear and mastoid air cells was measured in 55 temporal bones with normal ear drums from a Norwegian post mortem material. The measurements were made by an acoustic method and checked by means of fluid filling induced by vacuum pumping. As a measuring fluid X ray contrast medium was employed so that the degree of filling could be examined by X ray. The volume varied between approximately 2-22 cm<sup>3</sup> with an average round 6.5 cm<sup>3</sup>.

It is a common observation that both the extent and the composition of the mastoid air system can have great individual variations, and that it differs on the right and left sides in the same individual (Silbiger, 1950).

This is in accordance with our observations.

A system with many small air cells has a greater surface area than one with fewer, larger cells, when the volume is identical. It follows that a mastoid air cell system with larger cells should function better as a reservoir than a system with small cells.

Holmquist & Miller (1973) have studied the Eustachian tube and the middle ear function in the Rhesus monkey. They found that sudden pressure changes such as found in flying and diving, will stress the Eustachian tube function more in an individual with a big mastoid air cell system than in one with a smaller system.

Flisberg et al (1963) used Boyle's law for their clinical volume determinations of the air filled ear space.

Ingelstedt et al (1967) have earlier described a method for determination of volume of the air filled middle ear space in persons with intact ear drums.

This investigation has had two chief aims:

1 To measure the middle ear and mastoid air cell volumes in a Norwegian autopsy material.

2 To develop a method of measurement which can be employed clinically to patients with perforated ear drums without causing undue discomfort to the patient.

### MEASUREMENTS

The volume of an enclosed quantity of gas can be determined acoustically by measuring the change in pressure, i.e. sound pressure, when the volume is given a small harmonic (sinusoidal) change. The change in volume must occur so gradually that the change in pressure has time to adjust itself through the volume evenly. In other words, the sound frequency must be so low that the cross sections of the volume are small in relation to the wavelength.

It can then be shown that the sound pressure will be proportional to the relative change in volume and independent of the frequency.

$$p = \text{const} \cdot \frac{1}{V}$$

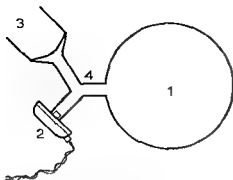


Fig 1 Arrangement for acoustical volume measurement.  
Volume to be measured 2 Hearing aid and telephone  
Microphone 4 Y tube

where  $p$  = sound pressure  
 $v$  = change of volume  
 $V$  = the volume to be determined

For the same change of volume in measuring two different volumes  $V_1$  and  $V_2$  the relation between corresponding sound pressures  $p_1$  and  $p_2$  will be

$$\frac{p_1}{p_2} = \frac{V_2}{V_1} \quad (2)$$

If  $V_1$  is a known reference volume  $V_2$  can be determined by

$$V_2 = V_1 \frac{p_1}{p_2} \quad (3)$$

Changes in volume can be obtained by using an ordinary magnetic hearing aid telephone. A constant excitation current to the telephone will cause the movement of the membrane to give a constant change of volume over a sufficiently large range of the measuring volume and frequency. The measuring arrangement is shown schematically in Fig 1

The supplementary volume  $V_e$  caused by the microphone, telephone and connecting tube can be determined by eq (2) by measuring the pressures  $p_1$  and  $p_2$  in two known volumes  $V_1$  and  $V_2$

$$\frac{V_2 + V_e}{V_1 + V_e} = \frac{p_1}{p_2}$$

1c

$$V_e = \frac{V_1 \frac{p_1}{p_2} - V_2}{1 - \frac{p_1}{p_2}}$$

Eq (3) can now be modified to

$$V_x = (V_r + V_e) \frac{p_r}{p_x} - V_e \quad (4)$$

where  $V_x$  = the volume to be determined  
 $V_r$  = the known reference volume  
 $p_x$  = the sound pressure in  $V_x$   
 $p_r$  = the sound pressure in  $V_r$

The frequency curve for the sound pressure is recorded to control that the response is flat at low frequencies. In the volume determinations the mean of the measured levels at 30 and 50 Hz was employed.

## MATERIAL

See tabular survey in the appendix (Table II)  
 Human temporal bones with normal ear drums were cleansed of soft tissue. A Y tube was placed in direct contact with the middle ear or mastoid air cells and cemented fast with Gnp dental cement. In some of the preparations, in addition to the placing of a direct tube, a tube was also inserted through a plug in the ear canal. In these preparations my-

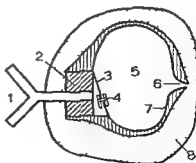


Fig 2 Y tube placed in the ear canal 1 Y tube 2 Neoprene plug 3 Ear drum 4 Ventilation tube 5 Middle ear 6 Eustachian tube 7 Medial bony wall of middle ear 8 Wax



myringotomy was performed, and in some cases a plastic ventilation tube was inserted.

The chance to get reliable measurements through the tube placed in direct contact with the cell system we wanted to measure, should be optimal.

But we also wanted to know whether we could get reliable measurements through a tube placed in the ear canal when there was a perforation in the ear drum. If so the method would satisfy the second chief aim of this investigation (qv). Since this tube ended in the ear canal it was not in direct contact with the volume we wanted to measure. Consequently we called this tube placing "indirect".

The following methods of tube insertion were employed:

### I Direct placing

- Through the tegmen tympani
- Through the bulbus superior venae jugularis internae
- Through the apex precessus mastoidei

### II Indirect placing

- In the ear canal in combination with myringotomy
- In the ear canal in combination with a ventilation tube in the ear drum (Fig 2)

The tube in the ear canal passed through a neoprene cellular rubber plug which filled the ear canal right up to the ear drum. (The small volume which might have remained between the plug and the ear drum in connection with these measurements constitutes so small a proportion of the total volume that it has been left out of account.)

The preparations were then dipped in melted paraffin wax several times until they were sealed within a thick airtight layer (Fig 3).

33 of the preparations were X rayed before measurement to obtain information concerning (a) the degree of pneumatization which affords an approximate idea of the volume (b) the size of the air cells (c) to examine the placing of the tubes (Fig 4) and (d) to ensure



Fig 3 Preparation No. 38 enclosed in wax. Y tube placed in the ear canal.

that the cell system was intact without pathological alteration or artefact.

All preparations were first measured acoustically. To remove possible fluid mass of the preparations, after the first acoustic measurement, were vacuum pumped before being subjected to a new acoustic measurement as a check. (See appendix.)

In order to check our acoustic measurements, all preparations were also measured by means of fluid filling. The air in the preparation was first pumped out and replaced with fluid from a graduated tube, so that the volume of fluid which was sucked in could be read off. The arrangement for measurement is shown schematically in Fig 5.

The air in the preparation, and the air because of the Y-type and additional tube is removed by means of a vacuum pump which is then closed at stopcock 3. Stopcock 4 is opened and the volume of fluid drawn in is read off from the graduated tube. The



Fig. 4. Pre-filling X-ray control. One tube through tegmen tympani, the other placed in the external ear canal. Internal porus acusticus and the cochlea are seen near the apex of the pyramid.

known volume  $V_x$  is determined by subtracting the extra volume  $V_e$

$$V_x = V_t - V_e \quad (5)$$

An air pressure of 1.5 torr was produced by pumping. The remaining air will therefore result in only about 2% too little fluid being drawn in. This is a negligible error. As measuring fluid was employed the X-ray contrast medium Isopaque Cerebral Nyco 280 mg I/ml diluted with 1 part contrast 2 parts water so that the degree of filling could be supervised by X-ray. Finally, the preparations were opened and checked under an operation microscope to ascertain whether there was any damage of the ear drum, the annular ligament and the membrana fenestra cochleae with possible leakage of the contrast fluid outside the air-filled cavities.

## RESULTS

All the primary measurements are given in the Appendix. The results are summarised in Table I to provide a synopsis and a basis for comparison of results. The volume distribution of 39 temporal bones measured acoustically after vacuum pumping is shown in Fig. 6.

## DISCUSSION

Littler (1965) reports, following Zalewski, that the breaking strength of ear drums of normal appearance in post mortem preparations is 0.4–3.0 atmospheres. The mean value for 111 ears was 1.6 atmospheres. In our experiments the middle ear pressure was reduced to approximately 1.5 torr (1 atmosphere = 760 torr). The ear drum will then move inward somewhat towards the middle ear, which will

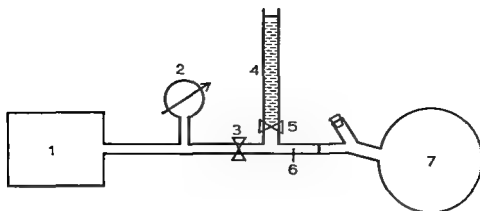


Fig 5 The arrangement for fluid filling 1 Vacuum pump 2 Pressure gauge 3 Stopcock 4 Graduated,

fluid filled tube 5 Stopcock 6 Connecting tube 7 Unknown volume (middle ear and mastoid air cells)

result in an inconsiderable reduction in volume there (Fig 7). The same reaction of the ear drum will meanwhile cause a relatively great change in volume in the small amount of air trapped outside the ear drum. This involves a relatively large decrease in pressure in this space also, simultaneously with the fall of pressure in the middle ear, and the difference in pressure across the ear drum will thus be comparatively small.

It might be supposed that the considerable fall in pressure in the middle ear might cause rupture of the annular ligament and/or the membrane of the fenestra cochleae. However, since the theoretical tensile strength of pure water exceeds 1000 atmospheres, the fluid in the inner ear cannot expand. Simultaneous re-

duction of the pressure upon the fenestra cochleae and the fenestra vestibuli will thus be unable to dislocate these outwards, so that they burst.

On opening the preparations and examining them under the operation microscope, it was also apparent that these structures had withstood the pumping. The ear drum had burst, however, in two of the preparations.

In the acoustic method the placing of the measuring tube through the ear canal has afforded very nearly the same results as water

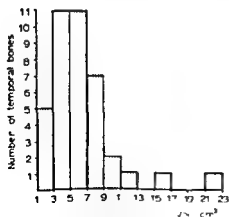


Fig 6 The volume distribution of temporal bones measured acoustically after vacuum pumping

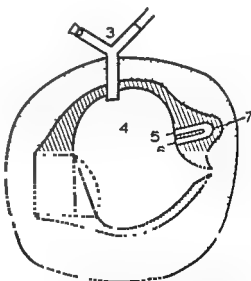


Fig 7 Vacuum pumping of middle ear 1 Small amount of air trapped outside the ear drum 2 Ear drum retracted somewhat inward during vacuum pumping 3 Y-tube connected to vacuum pump 4 Middle ear 5 Fenestra vestibuli 6 Fenestra cochleae 7 Cochlea

Table I Some different ways of presenting the results

Data definition	No of data	Volume of middle ear and airfilled spaces of mastoid process [cm <sup>3</sup> ]			
		Mean	Median	Quartiles	
				Lower	Upper
AM BP DIR	19	6.3	4.8	4.1	6.2
AM AP DIR	19	7.0	5.2	4.6	7.1
LM (I)	19	6.6	5.4	4.4	7.0
LM (10)	19	7.2	6.2	4.6	8.1
LM ( $\infty$ )	19	7.5	6.8	4.9	8.3
AM BP DIR	22	6.0	4.8	4.1	6.6
AM BP EC	22	6.1	4.8	4.1	6.7
AM AP DIR	23	6.4	5.1	4.3	6.9
AM AP EC	23	6.5	5.1	4.4	6.9
AM PB	51	6.1	4.9	3.7	7.6
♂	22	5.9	5.2	3.6	6.8
♀	27	6.0	4.9	3.9	7.9
LM ( $\infty$ )	46	6.9	6.0	4.5	8.3
♂	21	6.4	5.3	4.0	7.3
♀	23	7.2	6.8	4.9	9.7
AM BP DIR	34	5.9	4.9	3.9	6.6
AM AP DIR	34	6.5	5.2	4.4	7.6
LM ( $\infty$ )	34	6.8	6.0	4.3	8.1
AM BP DIR	39	5.6	4.8	3.6	6.7
AM AP DIR	39	6.2	5.1	3.9	7.6

Abbreviations AM Acoustical Method LM Liquid Method BP Before Pumping AP After Pumping EC measuring tube through Ear Canal DIR measuring tube inserted *DIR*ectly into volume (I) (10) ( $\infty$ ) indicate results with LM after approximately 1 10 seconds and a time long enough to give stationary values

the measuring tube is inserted directly into the middle ear or mastoid air cells (see point 2 in Table I). Volume seems to have little connection with sex (see points 3 and 4 in Table I).

In acoustic determination of volume, it was a necessary presupposition that measured sound pressure in the volume should be independent of the frequency within a limited frequency range. If this also proves to be the case in practice, it will provide a strong indication that the correct volume has been measured, viz., the volume of the middle ear itself and of the air filled mastoid cells. If, on the other hand the pressure increases with the frequency, that could indicate that more and more small part volumes are shut off from the rest of the total volume. This could be due to the mass inertness of the air, or through possible fluid membranes, which in the narrow

connective canals hinder the levelling out of pressure as the frequency increases.

Only a very few measurements have been omitted from our material on account of variation in the sound pressure within the actual frequency area (30–50 Hz).

The results derived from measurement by fluid filling are more difficult to interpret. The sucking in process happens rapidly at first, then more slowly. The individual variations in this process are wide. On the average, approximately 90% of the fluid will be drawn in during the first second, after 10 seconds, 96% will be drawn in, and the remainder in the course of 3–5 minutes. The total volume will, on average, be greater than when measured acoustically. This can be explained as being due to the opening of obstructed part volumes by the pumping out process. The obstruction can for example be caused by fluid membranes

Table II Results (volume measured in cm<sup>3</sup>)

Prep no	Sex	Age	Side	Acoustic method				Liquid method		
				Before pump		After pump		Filling time (sec)		
				Dir	E c	Dir	E c	1	10	∞
1	?	?	?	49						47
2	?	?	?	13						122
3	?	71	R	78						-
4	?	71	L	49						-
5	?	72	R	110						117
6	?	72	L	119						-
7	?	75	L	110						118
8	?	75	R	-						104
9	♂	82	R	78						67
10	♂	82	L	73						76
11	♂	74	R	-						-
12	♂	68	R	30						43
13	♂	68	L	36						47
14	♂	88	R	67	68	78	78			-
15	♂	88	L	40						53
16	♂	57	R	96		105				100
17	♂	57	L	93		102				96
18	♀	68	R	87		86				98
19	♀	68	L	52		70				63
20	♀	50	R	46		50				57
21	♀	50	L	79		85				83
22	♀	49	R	22	22	23	22			22
23	♀	49	L	25		36				39
24	♂	76	R	67		78				79
25	♂	69	R	-		-				-
26	♂	69	L	35		32				38
27	♂	69	R	57		57				56
28	♂	83	R	36		43				38
	♂	83	L	49	51	51	53			53
	♂	82	R	25		22		17	19	32
	♀	82	L	29		30				29
	♀	84	R	64	65	69	71	60	84	87
3	♂	65	L	18	18	22	22	20	22	25
34	♀	65	L	57		81		84	95	96
35	♀	95	R	33	33	36	31	26	31	-
36	♀	95	L	40	40	44	45	36	46	49
37	♂	54	R	47	48	51	52	45	45	46
38	♀	82	R	97	97	110	113	96	105	108
39	♀	82	L	141	142	151	154	117	122	126
40	♀	69	R	16	-	21	-	20	23	26
41	♀	69	L	13		19		-	-	-
42	♀	75	R	41	42	49	50	47	48	53
43	♂	75	L	68	68	69	66	-	-	-
44	♂	73	L	55	-	59	-	61	64	70
45	♀	84	L	59	61	58	64	58	61	69
46	♂	74	R	-	-	34	35	33	40	42
47	♂	74	L	41	41	53	55	52	55	57
48	♂	49	L	59	60	66	66	67	75	76
49	♀	64	L	72	74	73	75	72	77	79
50	♀	71	L	37	39	48	47	56	66	69
51	♂	75	L	192	190	210	220	219	223	224
52	♀	74	R	43	43	42	43	27	28	29
53	♀	74	L	41	41	46	46	45	49	49
54	♀	70	R	44	44	45	45	43	46	52
55	♀	70	L	48	48	50	50	45	62	67

Abbrev: Dir Direct E C Ear canal Brackets show paired preparations



Fig 8 Specimen No 41 anterior-posterior projection. Tomographic cut after contrast filling of this temporal bone visualize the internal acoustical porus, the vestibulum, the lateral and superior semicircular canal, the oval window, stapes, incus and Y tube through tegmen tympani. Contrast fluid filling is very poor. Possibly a thin shift of contrast fluid is located between the corpus of incus and the lateral semicircular canal.

fluid plugs in the connective canals between part volumes. Seen in this light, the agreement between the methods must be regarded satisfactory, and the fluid method with examination by X-ray of the degree of filling has confirmed the usefulness of the acoustic method.

Our measurements show a mean volume of approximately  $6.5 \text{ cm}^3$ . The values varied approximately between 2 and  $22 \text{ cm}^3$  (cf. Table I).

Silbiger (1950) found in his material from nasal mean values around  $9.5 \text{ cm}^3$  (varying from  $2.6$  to  $20 \text{ cm}^3$ ).

Zwislocki (1962) found in a limited material mean values around  $8.6 \text{ cm}^3$  ( $2.1$  to  $17.4 \text{ cm}^3$ ).

Flisberg et al. (1963) found variations from  $0$  to  $25 \text{ cm}^3$ .

Flisberg & Sigmond (1965) found in their material from Lund mean values around  $12.2 \text{ cm}^3$  in healthy ears.

Andreasson et al. (1976) have shown how the vascular bed (blood and lymph) in the mucous membrane influences on the volume of the middle ear when ambient and/or middle ear pressure is altered. This mechanism is thought to be of less importance in isolated temporal bones as in our experiments.

## APPENDIX

As appears in Table II, the material comprises altogether 55 temporal bones. 26 were from women, 27 from men. In the case of two preparations it was not noted whether they were from a man or a woman, left or right, the age or whether they formed a pair. 25 of the preparations were from the right side, 28 from the left. 12 of the female preparations were from the right side and 14 from the left. 13 of the male preparations were from the right side and 14 from the left. There were altogether 19 pairs of temporal bones, 10 female and 9 male. 15 of the preparations were not pairs, 6 right and 9 left. 6 of the single preparations were female (2 right and 4 left) and 9 male (4 right and 5 left). The ages varied from 49 to 95 years. The youngest woman was 49 years, the oldest 95. The youngest man was also 49, the oldest 88.

### Placing of tubes

Bulbus superior venae jugularis internae in 6 preparations, tegmen tympani in 48, through the ear canal together with a ventilation tube through the ear drum in 15 preparations, through the ear canal together with myringo-

tomia in 10 preparations, through the apex processus mastoidei in 2 preparations, through tegmen tympani and apex processus mastoidei in 1 preparation

Where the results of measurement have been omitted in Table II, some technical fault or other has arisen, e.g. calibration difficulties, leakage or poor contrast filling, such that the results of the measurement have been erroneous. X-ray examinations were carried out under fluoroscope supervision (16 mA, 45–55 kV). Use was made of antero-posterior, axialpyramidal, lateral and basal-axial projections for every preparation. 26 preparations were in addition tomographed to localize possible cells not properly filled by contrast fluid. For this purpose a Philips Polytome 100 with a hypocycloid movement was employed (2 mm cuts, 6 sec, 35 kV, 15 mA), with possible enlargement. The examination by X-ray of the preparations when filled with fluid often revealed a small bubble of air under the inner opening of the tube, but this was so small that it obviously had no significance in connection with the total volume. No cases of leakage of contrast fluid to the inner ear were detected.

Fig. 8 shows a preparation (no. 41 in Table II) where X-ray examination after filling with fluid shows so much air that we felt unable to use the results determined by the fluid method.

### ACKNOWLEDGEMENT

We are greatly indebted to Mr. Øyvind Lervik for invaluable technical assistance and to Miss Ellinor Kvam for doing all the writing.

### RESUMÉ

Le volume total de la caisse du tympan et des cavités mastoïdiennes a été mesuré sur 55 os temporaux avec des membranes du tympan normales et pratiqué sur une série d'autopsies norvégiennes. Les mesures ont été faites à l'aide

d'une méthode acoustique et contrôlées par remplissage de liquide après pompage à vide. Un moyen de contrôle radiologique a été utilisé permettant le contrôle radiologique du remplissage. Les volumes variaient de 2 à 6 cm<sup>3</sup> avec une moyenne se situant autour de 6.5 cm<sup>3</sup>.

### ZUSAMMENFASSUNG

Die Untersuchung umfaßt 55 Schläfenbeine mit normalem Trommelfell aus einem norwegischen Sektionsmaterial, bei denen das Gesamtvolumen von Mittelohr und Hörraum des Warzenfortsatzes gemessen wurde. Die Messungen erfolgten mittels einer akustischen Methode. Diese wurde kontrolliert, indem die zu untersuchenden Hohlräume vollständig entleert (Vakuum) und mit einer Flüssigkeit aufgefüllt wurden. Als Meßflußmittel war Röntgenkontrast verwendet, so daß der Füllungsgrad röntgenologisch kontrolliert werden konnte. Diese Volumina betrugen von 2–22 cm<sup>3</sup> mit einem Durchschnittswert von ca. 6.5 cm<sup>3</sup>.

### REFERENCES

- Andréasson L, Ingelstedt S, Ivarsson A, Jonson B & Tjernström O 1976 Pressure-dependent variation in volume of mucosal lining of the middle ear. *Acta Otolaryngol* (Stockh) 81: 442.
- Flisberg K, Ingelstedt S & Örtengren U 1963 Clinical volume determination of the air-filled ear space. *Acta Otolaryngol* (Stockh) Suppl. 182: 39.
- Flisberg K & Sigmond M 1965 The size of the middle ear cell system. *Acta Otolaryngol* (Stockh) 60: 21.
- Holmquist J & Miller J 1973 Eustachian tube and middle ear function in relation to volume changes in the mastoid air cell system. In *Proc. 4th World Conference on Otorhinolaryngology*, Venice, May 21–25, 1973 (ed. Arslan & V. Ricci).
- Ingelstedt S, Ivarsson A & Jonson B 1967 Mechanics of the human middle ear. *Acta Otolaryngol* (Stockh) Suppl. 228.
- Lütjens T S 1963 *The Physics of the Ear*. Pergamon Press, 326.
- Silbiger H 1950 Über das Ausmass der Mastoidmeninges bei Menschen. *Acta Anat* 11: 215.
- Zwislocki J 1962 Analyses of the middle-ear function. Part I: Input impedance. *J. Acoust. Soc. Am.* 34: 1514.

O I Mølvaer MD  
Dept of Otolaryngology  
University of Trondheim  
Trondheim  
Norway

## A CLINICAL PILOT STUDY ON PREFORMED AUTOLOGOUS OSSICLES I

A Tjellstrom J Lindstrom T Albrektsson P I Brånemark and O Hallen

*From the Laboratory of Experimental Biology Department of Anatomy and the  
Department of Otolaryngology University of Göteborg Göteborg Sweden*

(Received April 22 1977)

**Abstract** The study is part of a project aimed at obtaining autologous preformed transplant for reconstruction of effect ossicular chain. In animal experiments we found that bone could be produced in a titanium mould placed the tibia of the rabbit and dog. To find out whether this observation was applicable in man 5 patients with ossicular defects were selected. A titanium mould containing two chambers was placed in the proximal tibial metaphysis for 6 months. When the moulds were extracted bone was found in 7 of the 10 chambers. In 2 of these the bone was stable and suitable for ossiculoplasty. On histological examination the same picture as the animal experiments was found: an outer layer of cortical bone surrounding a system of spongy bone with marrow and haematopoietic cells. Microradiological examination verified the presence of mineralized bone tissue.

Most authorities are agreed that in clinical surgery the best bone for use as grafting material is that obtained from the patient's own tissue' (Burwell 1969).

Up until the 1950s surgery for chronic otitis media was chiefly adapted to meet the needs of pathology. In 1952 Wullstein and Zollner presented their first works on modern tympanoplasty. Later the introduction of the intact canal wall technique by Jansen (1968) made it possible to obtain a middle ear of normal width and also to reconstruct the ossicular chain in a new way.

There are two major problems connected with reconstructive ear surgery: to create an intact tympanic membrane and to achieve a sound conducting mechanism between the

drum and the inner ear. It is interesting to compare the evolution in myringoplasty with that concerning ossicular chain surgery.

Hall (1951) used rice paper to cover an eardrum perforation. Wullstein (1952) and Zollner (1952) suggested postauricular skin grafts. House (1960) described a technique using ear canal skin. The results of these trials were initially good but the various techniques all had drawbacks. More promising results were reported by Shea (1960) who used a vein graft. In 1964 Ortegren presented a study into the possibility of using autologous temporal fascia to repair a drum perforation and reported good results. Sheehy & Glasscock (1967) also reported excellent results with this type of graft. This material is easy to obtain and to handle and gives a high take rate regardless of the surgical technique used. Fascia seems to be the tissue of choice for most surgeons today. The choice of material used in myringoplasty has thus reached a stage where very few changes are called upon. In surgery of the ossicular chain the development has however, been quite different. There is no general agreement as to the best material for ossicular prostheses in spite of the many trials published during the last two decades. Wullstein (1955), Shea (1960), Austin (1965) and Shea (1974) presented studies with non biological implants. Studies presenting results with ho-



mologous ossicles have been published by Austin (1976), Smyth (1975), Sheehy (1975) and Palva (1976). Jansen (1972) and Smyth (1975) advocated homologous nasal septum cartilage for ossicular chain reconstruction. Autologous ossicles were used by Austin (1965), whereas Farnor (1960) tried cancellous bone and Ekvall (1973) used autologous cortical bone for reconstructive work.

The fact that experienced otologists concerned with the problems of ossiculoplasty have such diverging opinions on the material indicates that the ideal prosthesis has not yet been found. Some of the problems of getting a reconstructed ossicular chain to work in the long run are connected with resorption, bony fixation, extrusion, foreign body reaction etc. To avoid such problems the ideal prosthesis ought to be a fresh autologous ossicle with an intact vascular system and surrounded by a protective membrane. The idea of producing such a prosthesis came through an observation made in some animal experiments concerning integration of titanium screws in bone tissue (Brånemark et al., 1969). In a small hole in a titanium screw there was a tendency for growth of bone tissue to occur. This led to animal experiments presented in a previous paper (Hallén et al., 1976). In that study we found that bone could be produced in a titanium mould placed in the proximal tibial metaphysis of dogs.

To determine whether these experimental findings were applicable in man, the present clinical pilot study was carried out.

The aims of the study were

- 1 to find out whether bone is produced in a titanium mould placed in the proximal metaphysis of the tibia in man
- 2 to investigate whether bone produced in this way has biological properties that would make it suitable for use as an ossicle in the middle ear, and
- 3 to test whether such an ossicle could be handled and used as a prosthesis in ossiculoplasty.

It is not within the scope of this study to

draw any definite conclusions concerning the lasting value of this new type of ossicle graft. This can only be appraised after a long period of observation in several patients with meticulous follow up.

## MATERIAL

The patients were selected from the waiting list for ear surgery at the ENT Department, Sahlgren's Hospital, Göteborg, Sweden, according to the following criteria:

- 1 Age between 20 and 50
- 2 Known or strongly suspected ossicle damage
- 3 A dry ear without discharge for at least one year
- 4 No signs of otitis or cholesteatoma
- 5 A good speech discrimination score
- 6 Normal hearing in the contralateral ear
- 7 No general disease
- 8 A normal radiological examination of the proximal tibial metaphysis

Five patients were selected and pertinent clinical data for each patient are presented in Table I.

All patients were carefully informed about the possible advantages and also about the risks with this type of surgery. They were also informed that the procedure was part of an experimental clinical investigation. The study was approved by the Ethical Committee for Clinical Trials of the University of Göteborg, Sweden.

## METHOD

Five moulds were constructed out of titanium (ATi 24 Avesta Jernverk, Sweden) (Fig. 1). As in all reconstructive ear surgery the exact location of the ossicular defect was hard to predict, even in these cases. For this reason each mould was made with two chambers, one of them designed for the situation with an intact stapes and the other one for those cases in which only a foot plate remained (Fig. 2). The shape and size of the preformed ossicle

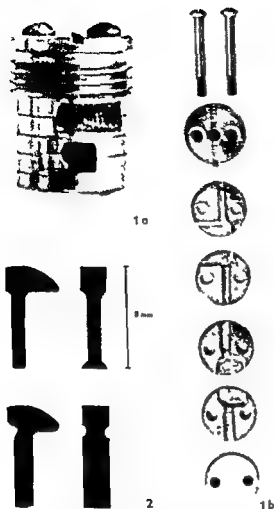


Fig 1 (a-b) The titanium mould assembled and taken apart

Fig 2 Shape and size of the chambers in the mould. Top figure meant for situation with just the foot plate left. Bottom figure meant for situation with an intact stapes

were based on the results found in an earlier study on the dimensions of the middle ear (Tjellstrom 1977). The moulds were made with a high degree of precision. Their surfaces were carefully polished with ground pumice stone and then washed in tap water. With the mould taken apart, it was then cleaned in five different baths of water under ultrasonic irradiation, treated with trichlorethylene in or

der to remove all fat and finally washed in absolute alcohol. After this cleaning procedure, the mould, still not assembled, was autoclaved on a titanium tray.






### *Surgical procedure I*

Under strict aseptic conditions the mould was placed in the left tibial metaphysis of the patient. Under local anaesthesia with 5 cc of 2% Xylocaine with epinephrine 1:100 000, a skin incision was made over the medial-anterior surface at the level of the tuberositas tibiae. A periosteal flap was lifted up and a hole was made in the cortical bone with a specially constructed trephine burr of the same diameter as the mould. These procedures were performed under generous saline irrigation to diminish heat trauma. The wall of the hole was then threaded with a tap. Some marrow was taken out and put into the chamber of the mould in the hope of facilitating osteogenesis. The mould was then placed in the threaded hole of the bone and secured with a titanium screw driver. This was also done under saline cooling. The periosteal flap was put back in place and adapted with catgut sutures. Haemostasis was established and the skin was sutured. To minimize the surgical trauma as much as possible, all surgical work was done very cautiously. A firm bandage was applied to the proximal tibia for one week after which the wound was inspected. The sutures were removed 12 days after surgery. The patients stayed at the hospital for 3 hours after the operation but were then allowed to move about and walk freely.

### *Surgical procedure II*

The second operation was planned according to the following schedule. Six months after the mould was placed in the tibia, the patient was admitted to the hospital for the ossiculoplasty. The operation was performed under general anaesthesia and the middle ear was exposed through the postauricular approach. The titanium mould was taken out from the tibia with specially designed instruments. In those

Table I Preoperative situation of the patients unfilled=defect

Patient	Sex	Age	Diseased ear	Drum	Ossicular defect	Pure tone audiogram	Speech discrimination score	A/B gap
SL	♀	43	sin	normal		43 dB	88%	25 dB
KO	♂	41	dx	atrophic but intact		56 dB	100%	20 dB
JO	♀	31	sin	dry central perforation		50 dB	100%	37 dB
KR	♂	46	sin	normal		78 dB	92%	33 dB
HK	♂	24	dx	normal		45 dB	100%	45 dB

instances in which bone suitable for ossiculoplasty was found, it was used for the reconstruction. Bone tissue not used in the surgical procedure was put into a transport medium for later histological studies. All handling of the bone was done in a glass container with the patient's own plasma. In those cases in which no usable bone was found in the mould a prosthesis of cortical bone was made.

During the surgical procedure a piece of cortical bone was taken out from the mastoid and trimmed to form an ossicle with a rounded butt under saline cooling in accordance with the usual routines for ossiculoplasties at our department. This bone was then treated and studied in the same way as the preformed one. In patients with a perforation of the tympanic membrane autologous temporal fascia was used as a drum graft underlay technique. Gelfoam<sup>®</sup> soaked in saline solution was put into the middle ear to support it. No Gelfoam<sup>®</sup> was allowed to come into contact with the new ossicle. The ear canal was filled with surgical rayon and pieces of acrylic cotton. The incision was closed with interrupted Polydec<sup>®</sup> sutures. One week after surgery the ear canal packing was removed.

## RESULTS

The findings in each patient are presented separately below and a schematic drawing of

the amount of bone found in each mould presented in Table II.

**Patient S L** In the wide part of the mould bone was found but the shaft consisted of a thin string of fibrous tissue. The bone produced had a cortical layer surrounded by fibroblasts and a marrow cavity in the centre. Because of the fibrous shafts, these bones could not be used for the ossiculoplasty. Instead, a piece of cortical bone from the mastoid was used as a connection between the foot plate and the handle of the malleus.

**Patient K O** Bone was found in the most superficially situated chamber but not in the one in the marrow cavity of the tibia (Fig 3).

Table II Schematic drawing of the amount of bone produced in the different patients unfilled=defect











Patient	Chamber closest to the cortical endosteum	Chamber with marrow cavity
S L		
K O		
JO		
K R		
H K		



Fig 3 A preformed autologous ossicle

Macroscopically, the ossicle had a cortical layer around spongy bone with marrow. It was stable and easy to handle in the reconstruction. In the other chamber no bone was found, only fibrous tissue.

**Patient J O** In this patient the same kind of bone was found as in the patient S L. A cortical shell of bone round a marrow space was produced in the wide ends of both chambers but in the narrow shafts only fibrous strings were found. It was not stable enough to be used for the ossiculoplasty. A malleus-stapes assembly as described by Austin & Glasscock was performed.

**Patient K R** Both chambers contained bone of the same appearance as in the other patients. However, in the chamber for the slimmest prosthesis there was a defect in the

middle of the shaft (Table II). Also in this patient the wider chamber had been placed most superficially.

**Patient H K** No bone was found in either of the chambers. They were filled with marrow surrounded by a fibrous tissue layer. In this case a piece of cortical bone was placed between the foot-plate and the handle of the malleus.

Four of the patients complained of pain from the implantation for some days and one of them had difficulty in walking freely for one week. These symptoms were not expected. After extraction of the moulds there was less discomfort. No signs of local infection were found after any of the surgical procedures.

In conclusion, bone was found in seven of the ten chambers. However, in only two of them had bone suitable for ossiculoplasty been formed. As shown in Table II, bone formation was more often observed in the superficial chambers than in the deeper ones. It should be noted, however, that the narrowest chambers were always placed deepest in the tibia.



Fig 4 Histological appearance of the bone produced. Bone trabeculae with filled osteocyte lacunae surrounding a haematopoietic marrow. An outer coating of mature connective tissue could be identified.  $\times 150$



Fig 5 Microcardiogram of a preformed autologous ossicle. An outer shell of mineralized bone tissue could be identified

## HISTOLOGY

The preformed osseous tissue that was not used in the middle ear of the patient was studied histologically and by microradiography.

The chamber contained bone trabeculae surrounding a haemopoietic marrow with some fat cells. On the outer side of the bone there was a 50–100  $\mu$ m wide zone of mature connective tissue. The bone trabeculae had oval shaped filled osteocyte lacunae. In some there were osteoblast seams (Fig 4).

Microradiological analysis shows an outer shell of mineralized trabecular bone tissue (Fig 5).

## DISCUSSION

In three out of the ten chambers no bone formation was detected, only blood and fibrous tissue being identified. In the remaining seven chambers bone tissue was found in two cases completely and in five cases incompletely filling the chambers. All bone produced had the same histological structure with an outer cortical layer and a central part consisting of cancellous bone and red bone marrow. On the surface of the bony cortex a layer of fibroblasts could be seen. In only two of the chambers was bone found in the entire shafts. Thus it seems most difficult to obtain a formation of stable bone in the narrowest part of the moulds. It was also found that the part

of the chamber where least bone was produced was at the same time the most central part of the mould with the longest distance to openings of the chambers. Little is known about osteogenesis under these special conditions but one possible explanation for the results might be that the chambers were too narrow.

An interesting finding was that more bone seemed to be formed in the chamber that was placed closest to the endosteal surface of the tibia. In animal experiments we were not able to find any differences in the capacity for bone formation when the moulds were placed deep in the marrow cavity close to the endosteum or in the bony cortex (Hallén et al 1976). However, it is known that the highest osteogenic activity is in the periosteal and the endosteal areas. It is also known that there are differences between species concerning osteogenic capacity, which might explain the differing results. The level of the mould in the tibia could be changed to overcome this situation. At the insertion the moulds were filled with red bone marrow and marrow haematoma. The demand on the body's capacity to form new bone tissue might however be too great. To have the body remodel cortical bone might be a better method than the procedure used in this study. Further studies will show whether or not this is true. Another factor that might be of importance is the time elapsed between implantation and extraction of the moulds. The time interval in the present study was 6 months based on the results from animal experiments. Perhaps this time was too long or too short for humans. The strong fibrous tissue in the shaft observed in some patients did not give any definite information as to whether there had originally been bone tissue which had undergone resorption. The lack of functional stress might have caused resorption of the bony tissue.

The present investigation has shown (a) that under certain conditions the human body can form bone tissue in a titanium mould placed in the proximal metaphysis of the tibia.

(b) that the macroscopic and microscopic appearance of bone produced in this way suggest that it may possess properties that ought to be of great value for use as a prosthesis in tympanoplasty, and

(c) that bone tissue of this type can be handled at operation without detracting from its major advantages

There is however, still much to be learnt about bone regeneration under special conditions. The results presented here indicate that it might be possible for the body to produce a preformed ossicle. Based on the present findings, the design of the next clinical pilot study will be modified

# ZUSAMMENFASSUNG

Im Rahmen eines Forschungsplanes versuchen wir ein autologes präformiertes Transplantat für Rekonstruktion einer defekten ossikulären Kette zu erreichen. In Tierexperimenten haben wir zeigen können, daß Bein in einer Titaniumform, die man in das Schienbein eines Kaninchens oder eines Hundes einbettet, gebildet werden kann. In der Absicht feststellen zu können, ob diese Beobachtung auch für Menschen gültig ist, wurden für die Untersuchung fünf Patienten mit Gehörknöchelchendefekten ausgewählt. In die linke proximale Tibiametaphyse wurde eine Form aus Titanium mit zwei Kammern für sechs Monate eingebaut. Als die Formen extrahiert wurden, wurden Knochengewebe in sieben von den zehn Kammern gefunden. Zwei Kammern waren mit Knochengewebe, daß sich für Ossikuloplastik eignete, ganz ausgefüllt. Die histologische Untersuchung ergab das gleiche Bild wie bei den Tierversuchen: ein Netzwerk von spongiosen Bein mit Mark und blutbildenden Zellen von kortikalem Bein umgeben. Die nachfolgende mikroradiologische Untersuchung bestätigte, daß es sich um mineralisiertes Knochengewebe handelte.

# REFERENCES

- Austin D F 1965 Present status of vein graft tympanoplasty. *Arch Otolaryngol* 81 20
- 1976 Transcatal tympanoplasty: a 15 year report. *Trans Am Acad Ophthalmol Otolaryngol* 82 30
- Bränemark P I, Breine U, Adell R, Hansson H O, Lindström J & Ohlsson A 1969 Intra osseous an-

- chorage of dental prostheses. *Scand J Plast Reconstr Surg* 3 81
- Burwell R G 1969 The fate of bone grafts. *Recent Advances in Orthopaedics* (ed A G Apley) chap 6 ■ 115 J & A Churchill Ltd London
- Ekvall L 1973 Total middle ear reconstruction. *Acta Otolaryngol* (Stockh) 75 279
- Farnor J B 1960 Ossicular repositioning and ossicular prosthesis in tympanoplasty. *Arch Otolaryngol* 71 443
- Hall A 1951 Prosthesis treatment and its healing effect on central perforations of the tympanic membrane. *Acta Otolaryngol* (Stockh) 39 136
- Hallén O, Bränemark P I, Lindström J & Tjellström A 1976 Preformed autologous ossicles. Experimental studies. *Acta Otolaryngol* (Stockh) 82 394
- House W F 1960 Myringoplasty. *Arch Otolaryngol* 71 399
- Jansen C 1968 The combined approach for tympanoplasty. *J Laryngol Otol* 82 779
- 1972 Methods of ossicular reconstruction. *Otolaryngologic Clinics of North America* 5 97
- Palva T 1976 Personal communication
- Shea J J 1960 Vein graft closure of eardrum perforations. *J Laryngol Otol* 74 358
- Shea J J & Honsy C A 1974 The use of Proplast in otologic surgery. *Laryngoscope* 84 1835
- Sheehy J L & Glasscock III M E 1967 Tympanic membrane grafting with temporalis fascia. *Arch Otolaryngol* 391
- Sheehy J L 1975 Otolologic Homografts. *Trans Am Acad Ophthalmol Otolaryngol* 80 ORL 37
- Smyth G D L 1975 Tympanic reconstruction long term results. 21st Yearsley lecture. Metropolitan Ear, Nose and Throat Hospital. Royal Society of Medicine
- Tjellström A 1977 Dimensions of importance in reconstructive ear surgery. *Acta Otolaryngol* (Stockh) in press
- Wullstein H 1952 Funktionelle Operationen im Mittelohr mit Hilfe des freien Spaltlappen Transplantates. *Arch Ohren Nasen Kehlkopfheilkd* 161 422
- 1955 Prognose und Resultat der Tympanoplastik. *Acta Otolaryngol* (Stockh) 45 440
- Zöllner F 1952 Plastische Eingriffe an den Labyrinthfenstern. *Arch Ohren Nasen Kehlkopfheilkd* 161 414
- Örten U 1964 Myringoplasty. Four years experience of temporal fascia grafts. *Acta Otolaryngol* (Stockh) Suppl 193

A Tjellström M D  
Dept of Otolaryngology  
Sahlgrenska Sjukhuset  
S-41345 Göteborg  
Sweden

## RELIABILITY OF BEKESY THRESHOLD TRACING IN IDENTIFICATION OF CARRIERS OF GENES FOR AN X-LINKED DISEASE WITH DEAFNESS

Agnete Parving

*From the Audiology Clinic ENT Department Gentofte University Hospital Hellerup Denmark*

(Received February 8 1977)

**Abstract** Seven identified carriers and 20 potential carriers

hearing impairment. The sensitivity of the method is poor. The specificity of the Békésy threshold tracing is high meaning that an absent dip cannot exclude the possibility of a subject being a carrier whereas a present dip can be regarded as an indication of a carrier. When comparing conventional octave audiometry and Békésy threshold tracing the latter method is found to be the more subtle in identifying carriers of genes for recessive deafness. There Békésy threshold tracing may be of help in the genetic counselling of potential carriers of genes for recessive deafness.

In epidemiological investigations it is a common finding that genetic factors account for hearing impairment in 50% of the cases (Barr et al., 1973, Fraser, 1974, Nance & Sweeney, 1975). The mode of transmission may be autosomal dominant, autosomal recessive, or sex-linked recessive. It has been estimated that approximately 75-80% of genetic hearing loss is caused by autosomal recessive genes, dominant and sex linked genes accounting for the rest (Nance & Sweeney 1975).

Genetic hearing losses may be congenital or may develop during childhood or early or late adulthood (Konigsmark 1971). They may also

be associated with abnormalities in other organ systems (Konigsmark 1969). An example of this is seen in Norrie's disease, also called progressive oculo acoustico cerebral dysplasia. This disease has formerly been described in ophthalmological (Warburg 1971) and in audiological (Parving & Warburg 1977) literature. The disease is well known as an X linked recessive trait, i.e. only affecting males with completely unaffected female carriers. The penetrance is complete, so that a carrier has a 50% risk of having a carrier daughter or an affected son.

Carriers of genes for recessive deafness cannot be identified by conventional pure tone audiometry (Wildervanck, 1957, Fraser, 1964). Carriers of X-linked recessive inherited hearing impairment may evidence minor audiologic abnormalities (Nance, 1971). In 1968 and again in 1976, Anderson & Wedenberg reported that normal hearing carriers of genes for deafness could be identified by means of Békésy audiometry. Among heterozygote carriers of genes for deafness, they found that 30% had small but distinct, peculiar 'dips' in their Békésy audiograms.

In an attempt to evaluate the reliability of the Békésy tracing method in identifying carriers of genes for X linked recessive hearing impairment, an investigation was performed on (1) normal hearing female subjects (2)

This work was supported by the Committee for Prevention of Blindness and Professor A. V. Lyskfeldt og Hustrus Legat.

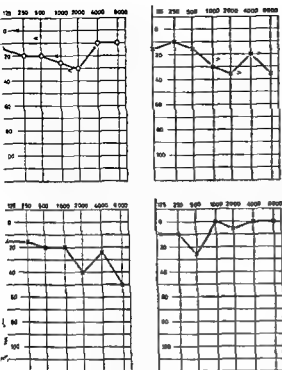


Fig. 1 Dip found by conventional pure tone octave audiometry in 3 known carriers and 1 potential carrier

known female carriers of Norrie's disease, and  
3) potential female carriers of Norrie's disease

## MATERIAL AND METHOD

Twenty nine female members of five families in which Norrie's disease occurred could be found. For various reasons 2 of them refused to participate in the investigation. The remaining 27 women ranged in age from 11 to 59 years, average 36.4 years. Being a mother or mother's mother to affected boys, 7 of these were identified to be carriers of the disease. Twenty subjects were potential carriers. Eight members of the latter group were sisters to an affected brother. Five women were sisters of mothers to affected sons (aunts) and 5 were cousins through their mother's sister. Two were sisters of a mother's mother. None of the sisters or cousins had affected sons.

Otосcopy and pure tone octave audiometry

were performed in all subjects. Thereafter, Bekesy audiometry was performed by means of a Bekesy accessory unit coupled to a Peters audiometer type Ap/6. The subjects were tested with both continuous and interrupted signals. In all tests the rate of attenuation change was 2.5 dB/sec. This attenuation rate was chosen because it gives no significant difference in excursion size with changing frequency (Siegenthaler, 1976). The signal changed the frequency at a speed control of 1/2 octave/minute. However, 3 subjects were examined with a changing frequency rate of 1 octave/minute due to age and discomfort in the sound proof hearing chamber. Two girls, aged 11 and 12 years, were not able to understand the Bekesy threshold tracing procedure. As the normal excursion size of Bekesy threshold tracing is 5–15 dB, the following criteria were used for the presence of 'dips'. The dip should be at least 20 dB HL and extend over at least 1 octave. As exogenous agents most frequently cause a hearing impairment in the frequency range above 3 kHz, the interval between 125 and 3 kHz was analysed.

Besides the family members, Bekesy audiometry was performed in 20 normal hearing female subjects. Their ages ranged from 23–69 years (average 37 years).

## RESULTS

All of the family members examined had normal otoscopy. The results of the pure tone audiometry showed 21 subjects had normal hearing. Six subjects had a hearing impairment. One of them had symmetrical hearing impairment in the high frequency area (sloping audiogram) due to age. One subject was monaurally deaf and had a sensorineural hearing loss of 60 dB HL in the left ear, which was probably due to a previous vascular catastrophe in the cochlea. Three known carriers and one potential carrier had audiograms showing a monaural hearing loss of 25–40 dB HL at only one or two frequencies, the 3 known carriers most pronounced at 2 kHz,



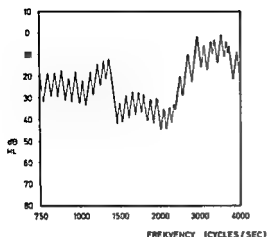


Fig 2 Typical dip seen in known carrier of Norrie's disease by Békésy threshold tracing (Only continuous signal shown)

and the potential carrier at 500 Hz (see Fig 1). No possible exogenous agent to account for this could be found.

Among the normal hearing female control subjects, no dips fulfilling the aforementioned criteria were found by the Békésy tracing procedure. Among known carriers, two had dips in the same ear centred around 2 kHz, where the peculiar dip was found by pure tone audiometry (see Fig 2). Five known carriers showed no dips in their Békésy threshold tracings, either by continuous or by interrupted sound stimulus. Three potential carriers had dips, 2 sisters to affected brothers centred around 2 kHz, one in both ears, the other in only one ear. In the last potential carrier the dip was centred at 500 Hz in the same ear as found by conventional audiometry. All the remaining family members had no dips fulfilling the criteria (see Table I). The lack of agreement in the number between the conventional audiometry and the Békésy threshold tracing in the known carrier group can only be explained by one subject's difficulties in understanding the Békésy tracing procedure. Therefore, this subject had pure tone audiometry performed with the Peters Audiometer at frequencies surrounding the 2 kHz "dip"-centre frequency found by conventional audiometry. The thresholds were determined by frequency intervals of 1 kHz between 1

and 4 kHz. This showed an increasing hearing loss from 1500 Hz through 1750 Hz up to 2 kHz and again decreasing through 2500 Hz and 3 kHz to nearly normal values at 4 kHz, thus confirming the dip configuration. This means that 42% of known carriers had dips by Békésy audiometry. In 2 girls, aged 11 and 12, this modified "tracing procedure" was performed to find a dip configuration centred around 2 kHz, but neither of them showed a tendency to dip configuration.

## DISCUSSION

If Békésy threshold tracing was theoretically the method of choice for finding all heterozygote carriers of genes for X linked recessive deafness, the following results from this investigation could be predicted. Dips present in 100% of known carriers, and 50% among potential carriers, calculated on the basis of the mode of transmission. However, in practice only 42% of known carriers and 15% of potential carriers showed dips" by Békésy threshold tracing. This means that the method is not sensitive enough to reveal the majority of carriers. This lack of sensitivity should be expected, as inherited recessive deafness is attributable to a great variety of different abnormal genes, and it would be difficult to imagine one single method being able to reveal all these abnormal genes.

No dips were found in the normal hearing female control subjects. In Anderson &

Table I Results of pure tone octave audiometry and Békésy threshold tracing in normals and carriers of Norrie's disease

	Normal	Known carriers	Potential carriers
Number	20	7	20
Number of subjects with dips in Békésy tracing	0	3 (42%)	3 (15%)
Number of subjects with dips in the conventional audiometry	0	3 (42%)	1 (5%)

Wedenberg's control group of 133 subjects of both sexes, dips were present in only 2% of the normal ears. This means that the specificity of Bekesy threshold tracing is at least 98% when regarding carriers of both autosomal and X linked recessive deafness. Regarding specificity, our results are in agreement with Anderson & Wedenberg, who found 30% showing dips in a population of parents to children with autosomal recessive inherited hearing loss. Therefore, it may be concluded that absence of "dip" in the Bekesy threshold tracing fulfilling the aforementioned criteria cannot exclude the possibility of the subject being a carrier. In contrast, the presence of a dip is indicative of a carrier of recessive autosomal or X linked deafness. Together with the family history, this may be of clinical importance.

The small one sided endogenous "dips" found by conventional pure tone octave audiometry in 42% of the known carriers and in 5% of the potential carriers are in disagreement with former investigations of carriers of sex linked recessive deafness (Fraser, 1964, Nance 1971). Fraser reports that carriers of X linked recessive deafness have normal hearing. In contrast Nance found in his investigation of stapedia foot plate fixation with perilymphatic gusher that all carriers of this X linked recessive hearing impairment had minor audiological abnormalities. The controversy between these two reports may be due to the fact that the carriers examined were not carriers of the same disease.

In this investigation the subjects were either 100% carriers or up to 50% risk carriers of genes for hearing impairment. Only 42% of the known carriers showed a dip at pure tone audiometry. This means that pure tone audiometry is not sensitive enough to exclude the possibility of the subject being a carrier of genes for hearing impairment. If all possible exogenous agents can be excluded, the finding of a dip may be regarded as an indication of a carrier. The dips may be specific for Norrie's disease.

In the group of potential carriers, two sisters with dips were found at Bekesy threshold tracing. The dip was not found by conventional octave audiometry. This means that when comparing pure tone octave audiometry and Bekesy threshold tracing, the latter method is the more subtle in finding carriers of genes for recessive deafness. However, investigations of a great number of known carriers of traits with known mode of transmission are necessary to evaluate further this method.

## ACKNOWLEDGEMENT

I wish to express my gratitude to Mette Warburg, M.D. head of the Copenhagen Eye Clinic for Mentally Retarded for referring patients with Norrie's disease.

## ZUSAMMENFASSUNG

Sieben identifizierte und 20 unidentifizierte Erbtäger von Norries Krankheit wurden mit Routine Audiometrie und Bekesy Audiometrie untersucht. Die Untersuchung unterstützt frühere Befunde mit Bekesy Audiometrie auf heterozygoten Trägern von Genen rezessiver Schwerhörigkeit. Die Sensitivität der Methode ist gering. Die Spezifität der Bekesy Audiometrie ist groß, dies bedeutet daß eine ausbleibende Besonderheit nicht die Möglichkeit ausschließen kann daß ein Patient ein Erbtäger ist, wohngegen eine vorkommenden Besonderheit als Anzeichen eines Erbtägers angesehen werden kann. Wenn man Routine-Audiometrie und Bekesy Audiometrie vergleicht findet man daß die Bekesy Audiometrie eine feiner reagierende Methode ist, wenn Träger von Genen rezessiver Schwerhörigkeit gefunden werden sollen. Es wird gefordert daß die Bekesy Audiometrie und die Familien-Anamnese zusammen helfen können, wenn unidentifizierte Träger von Genen rezessiver Schwerhörigkeit ge-  
neueschen Rat ersuchen.

## REFERENCES

- Anderson H & Wedenberg E 1968 Audiometric identification of normal hearing carriers of genes for deafness. *Acta Otolaryngol* (Stockh) 65 535
- 1976 Identification of normal hearing carriers of genes for deafness. *Acta Otolaryngol* (Stockh) 82 245
- Barr B, Anderson H & Wedenberg E 1973 Epidemiology of hearing loss in childhood. *Audiology* 12 426
- Fraser G H 1964 Profound childhood deafness. *J Med Genet* 1 118
- 1974 Epidemiology of profound childhood deafness. *Audiology* 13 335
- Kongsmark B W 1969 Hereditary deafness in man. *N Engl J Med* 281 713

- 1971 Hereditary deafness syndromes with onset in adult life *Audiology* 10 257
- Nance W E 1971 The principles and practice of genetic counseling *Ann Otol Rhinol Laryngol* 80 246
- Nance W E & Sweeney A 1975 Genetic factors in deafness of early life *Otolaryngologic Clinics of North America* 8 1 19
- Parving A & Warburg M 1977 Audiological findings in Norrie's disease *Audiology* In press
- Siegenthaler B M 1976 Reliability of Békésy excursion size among normal adults *J Aud Res* 15 111
- Warburg M 1975 Norrie's disease *Acta Ophthalmol* 217
- Wildervanck L S 1957 Audiometric examination: parents of children deaf from birth *Arch Otolaryngol* 65 280

Agnete Parving M D  
Audiology Clinic  
ENT Department  
Gentofte University Hospital  
DK 2900 Hellerup Denmark

## SUBJECTIVE DETECTION OF VERTICAL ACCELERATION A VELOCITY-DEPENDENT RESPONSE?

G Melvill Jones<sup>1</sup> and L R Young

From the Biotechnology Division Life Sciences NASA Ames Research Center  
Moffett Field CA and Man Vehicle Laboratory  
Department of Aeronautics and Astronautics Massachusetts Institute  
of Technology Cambridge MA USA

(Received Febr 24 1977)

**Abstract** Human subjective thresholds and directional sensitivity were investigated as a function of vertical linear acceleration with head erect. A hyperbolic ( $r = 0.94$ ) relation emerged between threshold latency and acceleration magnitude (range 0.005 to 0.06 g). This implies that detection was determined by attainment of a given velocity ( $21.6 \pm 2$  cm/sec) rather than the acceleration magnitude *per se*. Re-analysis of previous data from horizontal accelerations conducted with head erect and supine revealed similar hyperbolic relations ( $r = 0.98$  in both cases) with velocity constants of  $22.6 \pm 1.28$  and  $32.4 \pm 1.96$  cm/sec respectively. From these findings it is inferred that with head erect (i.e. normal attitude re gravity) the thresholds to predominantly utricular (horizontal accel.) and saccular (vert. accel.) stimulation were similar ( $P > 0.7$ ). However with head supine the saccular threshold was increased  $\approx$  approx. 1.5 $\times$ normal ( $P < 0.001$ ). The results also confirmed a previously reported difficulty in the subjective detection of the direction of vertical movement.

Subjective orientation estimates have long been known to depend on the orientation of the head relative to gravity, with the constant feature that the threshold of sensitivity to gravito-inertial stimulation tends to be least

This work was conducted at NASA Ames Research Center while Dr G Melvill Jones held a Senior Post Doctoral Award from the US National Academy of Science. Dr Young's participation was supported by NASA Grant NGR 22-009 701.

Present address: Director Aviation Medical Research Unit Dept of Physiology McGill University Montreal PQ Canada.

when the head is held in its normal erect position (Quix, 1925, Jongkees & Groen, 1946, Graybiel & Patterson, 1955, Graybiel & Clark, 1962, Schone, 1964). That this phenomenon is significantly dependent on the vestibular system is indicated by numerous observations. For example, the general phenomenon persists after minimizing non-vestibular somato-sensory cues through water immersion (Schone, 1964). Again, there is substantial underestimation of apparent tilt angle in labyrinthine defective subjects exposed to changes of the gravito-inertial vector using the human centrifuge (Graybiel & Clark, 1965).

Since these phenomena occur in the absence of dynamic stimulation of the canals, it may be inferred that there is a significant tendency for the sensitivity of specifically the vestibular otolith organ to become progressively reduced as its orientation relative to gravity deviates from normal. Indeed, for reasons detailed in the discussion, it seems that the phenomenon is only to be expected in the light of known structural and functional characteristics of that peripheral organ and its innervations.

Taken together, these observations strongly suggest that for proper comparison of orthogonal (X, Y, Z) response characteristics, the head should be held in its normal erect, at

Table 1 *Dependence of detectability of stimulus and directional assessment of acceleration upon stimulus acceleration magnitude*

Step acceleration magnitude $g$	0.005	0.007	0.009	0.011	0.015	0.020	0.040	0.06
% of undetected stimuli	28	17	13	13	3	■	0	0
% of wrong assessments of direction	30	28	38	26	29	23	34	■

titude relative to gravity. Experimental results meeting this requirement are available for horizontal acceleration with head erect (Travis & Dodge, 1928, Lansberg, 1954, Meiry, 1965, Niven et al., 1966, Young et al. 1966, Young & Meiry, 1968). However, although Z axis response has been studied during horizontal movement with the subject lying down (Jongkees & Groen, 1946, Walsh, 1961, 1962, Meiry, 1965, Niven et al., 1966), there is a dearth of modern data on the threshold response to vertical stimuli with head erect (Mach, 1875).

For this and other reasons mentioned below, Malcolm & Melvill Jones (1974) attempted to measure the subjective response of normally seated (i.e. head erect) human subjects to vertical accelerative movement using

AE (Canadian National Aeronautical Establishment) computer controlled helicopter and later a NASA (Ames Research Center) vertical movement simulator. Interpretation of their results was complicated by the unexpected finding that, in the absence of vision, subjects tended to become confused about the direction of movement which was not the case with Young & Mary's subjects moving in a horizontal plane. This difficulty seems the more surprising in view of the fact that good directional information is available in both primary afferent (Fernandez & Goldberg, 1976a and b, monkey) and central (Dauntton & Melvill Jones, 1973, cat) vestibular neural units responding to Z axis (body longitudinal axis) acceleration. Furthermore associated studies of vertical eye movement (Melvill Jones et al. 1976) and spinal motoneurone excitability (D Watt, personal communication 1977) during sinusoidal vertical movement revealed significant directional information in

the observed patterns of these forms of involuntary reflex response.

The present experiments set out (1) to measure normal threshold sensitivity to vertical acceleration with head erect, (2) to compare these results with those of Young & Meiry conducted in a horizontal plane and (3) to investigate further the peculiar difficulty of directional assessment referred to above. In addition, it transpired that, at least over the range of these experiments, the subjects' threshold of movement could be identified with a constant time integral of the imposed acceleration, independently of the magnitude of the acceleration, suggesting that the threshold sensation was a velocity-dependent response.

Similar methods to those of Young et al. (1966) have been employed in order to facilitate comparison of results.

## METHODS

All experiments were conducted on the NASA Ames Height Control Test Apparatus (HCTA) referred to above. Subjects were fixed in the seat of a blacked out cabin by a conventional aircraft restraining harness. In addition a restraining headband was adjusted to maintain head orientation so that a line joining the right orbital margin and external auditory meatus was tilted downwards 30° relative to the horizontal to bring the major plane of the utricular macula close to the true horizontal. The subject wore blackout goggles beneath which he maintained open eyes. The right hand was located on a light weight lever with three position switch, the mid position

presenting zero response and the up and down positions signalling a subjective sensation of the direction of acceleration. Ear muffs containing earphones permitted communication with the remote control cabin and attenuation of external auditory cues. Provision was made to use white noise for masking troublesome external sounds but in practice this proved unnecessary.

The additional head harness also served to avoid potential accessory cues from the canals due to pitching angular movements of the head potentially induced by the vertical linear accelerations with head tilted forwards. The effectiveness of this restraint was verified by means of a small, sensitive, pitch detecting oscilloscope mounted on a dental biteboard. Test runs with 3 subjects exposed to the whole range of the experiment showed that angular head movements were usually undetectable and never exceeded  $\pm 0.5^\circ$ .

The main experimental series employed 8 adult subjects with no clinical abnormality. One individual held a private pilot's licence but was not currently flying. None of the others was an aviator. They were each exposed on four occasions to each of eight magnitudes of step change in vertical acceleration. Acceleration magnitudes were all very low, ranging from 0.005 g to 0.06 g as shown in the top row of Table 1. The distribution of acceleration magnitudes was chosen to concentrate recordings in that part of the data set where the most rapid change of response latency with acceleration was expected from the previous horizontal acceleration data of Merry (1965). An 8x8 Latin square design permitted exclusion of learning effects and also a determination of whether practice during the experiment led to significant shortening of response latency.

All subjects were practised over the range of the experiment and informed of their performance during these runs. They were required to flick the indicator switch up or down as soon as possible after sensing the acceleration and according to the sensed direction of move-



Fig. 1. Records of stimulus (head acceleration) and response (subjective response) from a single test run. The initial acceleration (A) was always 0.005 g either up or down. The plateau velocity (B) was always 2 ft/sec either up or down. In this example an upward acceleration of 0.04 g was imposed after 10 sec of plateau velocity and correctly identified with a latency of 1.1 sec.

ment. Practice was continued until subjects were satisfied they knew what to do.

A potentially complicating factor was static friction of the cab in its track, which could produce a detectable jolt on commencing an acceleration from rest. Consequently all test accelerations were begun at randomly chosen times after achieving a steady linear velocity of 2 ft/sec, which in turn was always attained by means of the lowest controllable linear acceleration, namely 0.005 g. However, since cab movement was inevitably associated with some vibration, this procedure necessitated avoidance of a simple relation between direction of acceleration and any sensed increase or decrease in vibration. This was achieved by balancing the occasions when a given direction of acceleration stimulus would be associated with increasing or decreasing vibration.

Vertical acceleration was recorded from two sets of linear accelerometers, one installed on the cab and a two-dimensional linear accelerometer fixed firmly to the scalp. The head mounting was arranged so that one degree of freedom paralleled the earth horizontal in a fore-aft direction when the head was tilted  $30^\circ$  downwards and forwards. The orthogonal axis was aligned with the true vertical. The system allowed remote checking of the correctness of head position before each run, as well as readjustment of head position in the head harness when this proved necessary after rests between runs. Simultaneous recording of cab

and vertical head accelerometers showed that, there were no significant differences between the outputs of cab- and head mounted accelerometer systems. Also recorded in parallel with these outputs was the subject's switch position and relevant system parameters such as the servo command voltage, safety limiting control outputs and actual cab position derived from a track potentiometer.

## RESULTS

### Threshold characteristics

Fig 1 represents an original record obtained from a single test run. The upper trace is recorded from the vertically oriented linear accelerometer mounted on the head. Starting from rest, there was an initial period of very low upward acceleration ( $A$ ) at the standard value of  $0.005 g$  ( $0.16 \text{ ft/sec}^2$ ) until attainment of a steady, or plateau, upward velocity of  $2 \text{ ft/sec}$ . Then, after a randomly chosen duration of between 4 and 10 sec ( $B$ ) the test acceleration was applied (upwards in this example) and maintained ( $C$ ) until after the subject had registered his response by flicking the switch up or down (up in this example). The response latency was assessed as the time between initiation of the recorded test acceleration and the registration of subjective response. In practice all records were tape recorded and these latencies were measured from records played back on a suitably expanded time scale.

Fig 2 shows the mean values of latency ( $\pm$ Standard Error) obtained in this way for all subjects and all runs at each of the eight acceleration magnitudes. The values shown in this figure are independent of whether the subject made a correct or incorrect assessment of direction and, of course, only those occasions when responses were indicated contribute to the curve. In practice and as will be described below, all subjects responded on all possible occasions at acceleration magnitudes above  $0.015 g$ . However, as shown in the mid-

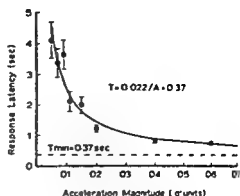


Fig 2 Dependence of response latency upon acceleration magnitude. The continuous curve shows calculated regression hyperbola which best fits these. Brackets give standard error of the means ( $N=8$  subjects).  $T$ =total response latency,  $A$ =step acceleration in  $g$ ,  $T_{\min}$ =calculated asymptote for the present results.

dle row of Table 1, and as is to be expected progressively fewer test accelerations were detected as acceleration magnitudes decreased below this value.

The curve drawn in Fig 2 shows the calculated least squares regression line fitted to the average latencies according to the hyperbolic relation

$$T = B/A + T_{\min} \quad (\text{for } A > 0.005 g)$$

where

$T$ =mean measured response latency (sec)  
 $A$ =step acceleration magnitude ( $g$ ),  $T_{\min}$ =reaction time independent of  $A$ ,  $B$ =slope of the regression line when plotting  $T$  against  $1/A$ .

The close fit of this calculated regression curve ( $r=0.94$ ) implies that the value of  $B$  in eq (1) represents a meaningful constant. An important feature of this conclusion is that the constant has the dimensions of linear velocity as is evident in the alternative form of the equation

$$B = (T - T_{\min})A$$

From this observation the significant inference may be drawn that over the whole range of this experiment, the value  $B$  presents a consistent threshold linear velocity which had to be attained before generation

sensation of the changed movement. The calculated value of this velocity ( $B$ ) for the regression curve of Fig. 2 is  $0.022 \text{ g sec}$  ( $=0.71 \text{ sec}=21.6 \text{ cm/sec}$ ). Amongst the 8 subjects tested, the individual calculated values of  $B$  ranged from 14.8 to 27.0  $\text{cm/sec}$  ( $S.D. \pm 5.3$ ).  $T_{\min}$  in eq. (1) and (2) is interpreted as the instant residual reaction time for initiation of mechanical movement of the "up-down" lever for perception of changed movement. The calculated value of this residual reaction time,  $T_{\min}$ , is 0.37 sec. This value is shown graphically as the dashed straight line in Fig. 2, representing an asymptotic limit of the calculated hyperbolic regression curve.

*Comparison with previous 'horizontal' data*  
In order to compare these results with the "horizontal" data of Young et al. (1966), their results have been refitted with "least squares" hyperbolic regression curves of the form shown in Fig. 2. In both instances, remarkably close fits were obtained, the calculated correlation coefficient,  $r$ , being 0.98 for both their erect and supine conditions. Also, as with the present results, meaningful values of  $T_{\min}$  were obtained (0.7 and 0.6 sec respectively) although in both instances the actual values were somewhat longer than the corresponding value of 0.4 sec from our results.

Graphical comparison of results is presented in Fig. 3. Here the ordinate gives response times for subjective detection of acceleration after subtraction of the respective minimum reaction time ( $T_{\min}$ ) so that all curves begin at the same origin. The abscissa gives the inverse of stimulus magnitude, expressed in non-dimensional units of  $\text{g}^{-1}$ . Plotted in this way the hyperbolic relation of Fig. 2 becomes a linear one in which, according to the expression in eq. (2), the slope  $B$  numerically determines the constant velocity threshold suggested above. The figure shows calculated linear regression lines for the three sets of results. The continuous line depicts the same line as that plotted in Fig. 2 and represents the

results from the present experiment. The corresponding data from Young & Meiry's experiments conducted in a horizontal plane with subjects seated erect and lying supine are shown as the dashed line and the dash-dot-dash lines respectively.

The first notable feature is the near superposition of results for vertical and horizontal stimuli with the head held in its *normal orientation* relative to gravity. The two lines are statistically indistinguishable ( $P > 0.7$ ), the respective values of the coefficient  $B$  being  $21.6 \pm S.E. 2.65$  and  $22.6 \pm S.E. 1.28 \text{ cm/sec}$ . The close conformity of the two data sets is particularly striking bearing in mind the fact that they were obtained from different experiments using different subjects, equipment and directions of acceleration. It seems reasonable to conclude that, taken together, they represent the normal threshold of response to linear acceleration over the low amplitude range of these experiments for vertical (up-down) and horizontal (fore-aft) directions of movement.

In marked contrast, the results from Young & Meiry's subjects exposed to horizontal acceleration in a supine attitude yielded a considerably higher value of  $B = 32.4 \pm S.E. 1.96 \text{ cm/sec}$  for the "velocity" constant ( $P < 0.001$ ). In view of considerations mentioned in the introduction as well as additional evidence discussed below, it is inferred that this difference is primarily due to the  $90^\circ$  deflexion of the head's Z axis from the gravitation vertical.

#### *Directional sensitivity*

The results in Fig. 3 show that, with head erect, there was no significant difference in threshold sensitivity to low amplitude acceleration in horizontal and vertical directions. However, whereas the horizontally accelerated subjects could effectively estimate the directional component of the stimulus, Table I shows that this was not so for our vertically accelerated subjects. The upper row of the table gives the acceleration magnitudes employed in these experiments. The middle row



shows that, as expected, the percentage of failures to detect the presence of an acceleration decreased rapidly to zero as the magnitude of acceleration increased. However, the bottom row shows that the percentage of incorrect assessments of the direction of acceleration remained essentially constant at about 30%, and was therefore independent of acceleration magnitude over the entire range of experiment. Thus, in line with the previous findings of Malcolm & Melvill Jones (1974) these results appear to demonstrate a specific difficulty in detecting the direction of vertically imposed movement with the head in its normal erect position.

#### Additional observations

The statistical design of these experiments permits investigation of effects due to (a) practice, (b) up going versus down going accelerations and (c) increasing versus decreasing levels of vibration. None of these influences produced statistically significant effects.

### DISCUSSION

threshold sensitivity. A velocity  
t = response

An unexpected feature of the results is the closeness with which the latency-amplitude plot of Fig. 2 can be fitted by a hyperbolic curve relating response latency to the inverse of the imposed acceleration. As pointed out in Results, this implies that over the whole experimental range the perception of movement was on average first sensed only after attainment of a fixed linear velocity  $B$  in eqs (1) and (2). The inference is substantiated by the additional finding that re-analysis of previous results obtained in a similar way, but different circumstances, by Young et al. (1966) yielded equally close fits to hyperbolic relationships. There seems little doubt that in these experiments it was the attainment of a threshold velocity, rather than the acceleration *per se*, that determined the time to detect

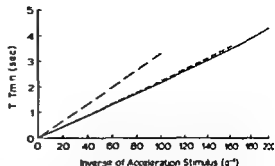


Fig. 3. Comparison of results in Fig. 2 (—) with that of Young, Meiry & Li (1966) for horizontal acceleration with head erect (—) and in supine position (---) after subtraction of the calculated minimum reaction times ( $T_{min}$  in eqs (1) and (2)). Note that response times (seconds) (ordinate) is plotted against the inverse of acceleration stimulus ( $g^{-1}$ ). The lines are calculated "least squares" regression lines for each data set with respective correlation coefficients ( $r$ ) of 0.98 and 0.95.

tion of changed movement. The finding is closely akin to that associated with the well-known "Mulder product" (van Egmond et al. 1949) for the semicircular canals. Thus dynamic characteristics of the semicircular canals can be approximated by an integrating angular accelerometer over a wide range of brief acceleration stimuli, such that the product of acceleration and time-to-detect is constant (1.5–2.0°/sec). However, neither direct measurement of otolith end-organ mechanics (De Vries, 1950) nor system analysis of identified primary afferent response (Fernandez & Goldberg, 1976b) points to a peripheral vestibular origin for this well-defined integrative characteristic. Analysis of peripheral otolith units indicates the presence of a first order lag operator which could be associated with the mechanics of otolithic membrane displacement. However, the relevant time constant determined by Fernandez & Goldberg (median 16 ms for regular units and 25 ms for irregular units) are more than two orders of magnitude too small to account for acceleration integration implied by the present experiment. Furthermore, their illustration of regular and irregular unit responses to step duration force trapezoids (approximating

present acceleration profiles) show a rapid rise in unit activity following force onset, and no evidence of any long time constant integration of acceleration in the end organ.

This raises the important question, to what extent can the perceptual response to these very low level accelerations be attributed to stimulation of the vestibular sensory system? The matter was touched upon in the introduction, where studies were quoted in which subjective sensations of change in direction of the perceived vertical were retained after minimisation of somatosensory cues by water immersion but were impaired in labyrinthine defective subjects. Furthermore, Young & Graybiel (unpublished) found that labyrinthine defective subjects had 8–15 times the normal threshold for detection of horizontal linear acceleration, which is similar to the earlier finding of Walsh (1961). Moreover, that the central neural vestibular response would be sufficiently sensitive to detect the low accelerations employed in these experiments is indicated by the very low thresholds ( $>0.005$  g) reported for single acceleration dependent central neural units in the cat (Melvill Jones & Milsum, 1969; Dauntun & Melvill Jones, 1973). Theoretical calculation of acceleration thresholds based upon the signal to noise ratio in the individual otolith nerve fibres are also consistent with the observed acceleration thresholds (Ormsby, 1974). Thus it seems that the very low acceleration amplitudes employed here, extending down to the absolute threshold of sensory perception (around  $0.005$  g, see Fig. 2) would be unlikely to generate detectable bodily somatosensory cues but likely to activate suprathreshold response in the specially adapted vestibular organ.

#### *Implications concerning utricular and saccular components of response*

Insofar as the above results can be ascribed primarily to vestibular stimulation, the close similarity between head erect responses to horizontal and vertical accelerations implies similar response characteristics in utricular

and saccular components of the otolith organs. Thus both the direction specificity of hair cell orientation (e.g. Spoendlin, 1966; Lindeman, 1969; Lowenstein & Wersäll 1959; Flock, 1964) and more recently the separate recording of primary afferent neurones from saccular and utricular maculae of the monkey (Fernandez et al., 1972; Fernandez & Goldberg, 1976a) show that, with head erect, horizontal and vertical movements predominantly stimulate utricular and saccular end organs respectively.

Additional results from the latter authors also have a direct bearing on the marked difference between threshold sensitivities determined in the direction of predominant saccular stimulation with the sacculus in its normal (head erect) attitude and tilted  $90^\circ$  relative to this attitude (subject supine). The difference is seen by comparison of the continuous and the dash-dot dash lines in Fig. 3. As stated in results, the respective slopes of these lines are  $22.6$  and  $32.4$  cm/sec, this difference being statistically highly significant. From this finding it may be inferred that the threshold of sensitivity to saccular stimulation is significantly lower when in its normal orientation relative to gravity than when tilted  $90^\circ$  away from that orientation. In the same context the above authors noted that saccular-dependent vestibular primary afferents responding to  $+Z$  (upwards re head) and  $-Z$  force vectors have similar steady state discharges with head erect, but the same populations have significantly different steady state values when the  $Z$  axis of the head is horizontal. Assuming it is the differential firing rate between  $+Z$  and  $-Z$  units which constitutes the meaningful signal, then a given change of signal effected by a given acceleration would constitute a smaller proportion of the static differential signal with the  $Z$  axis horizontal than when in its usual vertical orientation. According to Weber's principle the just noticeable change (i.e. threshold) would then be associated with a larger stimulus when the  $Z$  axis was horizontal.

Assume that the ratio of minimal detectable change in firing rate ( $\Delta f$ ) is proportional to the spontaneous rate ( $f_0$ )

$$\frac{\Delta f}{f_0} = K$$

Assume further that the spontaneous firing rate for +Z saccular units ( $f_{0+}$ ) is raised and that for -Z units ( $f_{0-}$ ) reduced when the Z-axis is horizontal, and that the signal ( $\mu$ ) which must be detected is the difference between +Z ( $f_+$ ) and -Z ( $f_-$ ) unit rates, minus the difference in their spontaneous rates ( $f_{0+} - f_{0-}$ ). The variances of the difference in signals ( $\mu$ ) is the sum of the variance of the +Z and -Z rates ( $f_+, f_-$ ), which in turn are assumed proportional to their spontaneous rates. Consequently, an increase in  $f_{0+}$ , even though accompanied by a decrease in  $f_{0-}$ , will raise the standard deviation in the net signal, and increase the threshold for detection of acceleration for Z-axis horizontal, relative to Z-axis vertical.

### Directional sensitivity

Turning to the question of our subjects' apparent insensitivity to the direction of vertical acceleration, the possibility arises that they were responding simply to some form of vibration of vibration. A number of features, however, suggest this is an unlikely explanation. First, if the subjective response to acceleration carried good directional information, then since the sensory signal would presumably increase with increasing acceleration magnitude, one might expect there would then be a corresponding reduction of the directional uncertainty, which there was not. Moreover, similarity between the present data and those of Young et al. (1966) over the range of experiment is too close to have been fortuitous and yet in marked contrast to the present results, Merz's subjects who were exposed to horizontal acceleration were able to detect direction with confidence.

Thus the characteristic of directional uncertainty seems to have been associated spe-

cifically with the imposition of linear acceleration in a vertical direction, parallel to gravity. This direction is the only one associated with no relative rotation of the gravito-inertial acceleration vector. Possibly, therefore the presence of this variable is a contributory factor (Benson & Bodin, 1966; Correia & Guedes, 1966). Perhaps also the difficulty in perceptual interpretation of the central message could be associated with a difference in the "need to know" about the direction of acceleration in horizontal and vertical directions. In this connection it is interesting to note that recent results of Vidic et al. (1976) have demonstrated differences between the oculomotor reflex responses to accelerations stimulated primarily saccular and utricular otolith organs, despite the similarity of subject sensitivity thresholds revealed in the present study.

### ACKNOWLEDGEMENTS

The expert technical assistance at NASA Ames Research Center which made these experiments possible is fully acknowledged as is the patient and co-operative contributions of our human subjects.

### ZUSAMMENFASSUNG

Subjektive Schwellen und Richtungsempfindlichkeit für vertikale lineare Beschleunigungen wurden in aufrechter Kopfhaltung untersucht. Zwischen Schwellenleistung und Größe der Beschleunigung wurde eine hyperbolische Beziehung ( $r=0.94$ ) gefunden (im Bereich zwischen 0.06 und 0.06 g). Dies bedeutet, daß die Schwelle eben so hoch ist, wie die absolute Geschwindigkeit (21.6 ± 2.63 cm/sec). Eine erneute Analyse von früheren Daten von horizontaler Beschleunigung mit aufrechter Kopfhaltung ergab eine ähnliche hyperbolische Beziehung ( $r=0.98$  in beiden Fällen) mit Geschwindigkeitskonstanten von jeweils 22.6 ± 1.28 und 32.4 ± 1.96 cm/sec. Diese Ergebnisse zeigen, daß bei aufrechter Kopfhaltung (also in der Normalposition bezüglich Erdanziehung) die Schwellen bei vorwiegend sinusförmiger Utriculi (horizontale Beschleunigung) und Sacculi (vertikale Beschleunigung) ähnlich sind ( $P>0.7$ ). In Rückenlage ist die Sacculus Schwelle jedoch auf ungefähr das Doppelte über den Normalwert erhöht ( $P<0.001$ ). Diese Resultate bestätigen die auch schon früher beschriebene Unsicherheit im Erkennen der Richtung vertikaler Beschleunigungen.

## REFERENCES

- Arson A J & Bodin M A 1966 Interaction of linear and angular acceleration on vertical receptors in man *Aerospace Med* 37 144
- Attia M J & Guedry F E 1966 Modification of vestibular responses as a function of rate of rotation about an earth horizontal axis *Acta Otolaryngol* (Stockh) 62 797
- Buntin N & Melvill Jones G 1973 Directional representation of horizontal and vertical acceleration in the neural activity of cat vestibular nuclei *Proc Aerospace Med Ass Ann Sci Mtg Las Vegas* p 144
- Also in *Comparison of brainstem neural responses to vertical and horizontal linear accelerations*. Progress report to NASA Ames Research Center p 29 1973
- Busch H I 1950 The mechanics of the labyrinth otoliths *Acta Otolaryngol* (Stockh) 38 263
- Clark A 1964 Structure of the macula utricula with special reference to directional interplay of sensory response as revealed by morphological polarisation *J Cell Biol* 22 413
- Cernandez C & Goldberg J M 1976a Physiology of peripheral neurones innervating otolith organs of the squirrel monkey I Response to static tilts and long duration centrifugal force *J Neurophysiol* 39 970
- 1976b Physiology of peripheral neurones innervating otolith organs of the squirrel monkey III Response dynamics *J Neurophysiol* 39 996
- Cernandez C, Goldberg J M & Abend W 1972 Response to static tilts of peripheral neurons innervating otolith organs in the squirrel monkey *J Neurophysiol* 35 978
- Graybiel A & Clark B 1962 Perception of the horizontal or vertical with the head upright on the side and inverted under static conditions and during exposure to centripetal force *Aerospace Med* 33 147
- 1965 The validity of the oculographic illusion as a specific indicator of otolith function *Aerospace Med* 36 1173
- Graybiel A & Patterson J L Jr 1955 Thresholds of stimulation of the otolith organs as indicated by the oculogravic illusion *J Appl Physiol* 7 666
- Jongkees L B W & Groen J J 1946 The nature of the vestibular stimulus *J Laryngol Otol* 61 529
- Lansberg, M. J. 1954, *Untersuchungen über die Funktionen in der labyrinthischen Funktion* *Aeromed Acta* (Soesterberg) 3 209
- Landman H H 1969 Studies on the morphology of the sensory regions of the vestibular apparatus. In *Advances in Anatomy Embryology* p 1 Springer Berlin-Heidelberg New York
- Lowenstein O & Wersall J 1959 A functional interpretation of the electromicroscopic structure of the sensory hairs in the cristae of the elasmobranch *Raja Clavata* in terms of directional sensitivity *Nature* (Lond) 184 1807
- Mach E 1875 *Grundlinien der Lehre von den Bewegungsempfindungen* (pp 31 32) Engelmann Leipzig also Bonset Amsterdam 1967
- Malcolm R & Melvill Jones G 1974 Erroneous perception of vertical motion by humans seated in the upright position *Acta Otolaryngol* (Stockh) 77 274
- Meury J 1965 The vestibular system and human dynamic space orientation Sc D Thesis Massachusetts Institute of Technology NASA CR-628
- Melvill Jones G & Malsum J H 1969 Neural response of the vestibular system to translational acceleration *Proc Conf on Systems Analysis in Neurophysiology Brainerd Minn Suppl* p 8
- Melvill Jones G, Rolph R & Downing G H 1976 Comparison of human subjective and oculomotor responses to sinusoidal vertical linear acceleration DRB Aviation Medical Research Unit Reports Vol V p 256 Report No DR 225
- Niven J I, Hixon W C & Correia M J 1966 Elicitation of horizontal nystagmus by periodic linear acceleration *Acta Otolaryngol* (Stockh) 62 427
- Ormsby C 1974 Model of human dynamic orientation Ph D Thesis Massachusetts Institute of Technology Cambridge Mass
- Quinn F H 1925 Function of the vestibular organ and clinical examination of the otolithic apparatus *J Laryngol Otol* 40 425
- Schone H 1964 On the role of gravity in human spatial orientation *Aerospace Med* 35 764
- Spoendlin H 1966 Ultra structure of the vestibular sense organ. In *The Vestibular System and Its Diseases* (ed R J Wolfson) University of Pennsylvania Press Philadelphia p 39
- Travis R C & Dodge H 1978 Experimental analysis of the sensorimotor consequences of passive oscillation—rotary and rectilinear *Psych Mon* 38 1
- van Egmond A A G, Groen J J & Jongkees L B W 1949 The mechanics of the semicircular canal *J Physiol* (Lond) 110 1
- Vidic T R, Barlow J S, Oman C M, Tole J R, Weiss A D & Young L R 1976 Human eye tracking during vertical and horizontal motion *Neuroscience Abstracts* (Revised) No 1536
- Walsh E G 1961 Role of the vestibular apparatus in the perception of motion on a parallel swing *J Physiol* (Lond) 155 506
- 1967 The perception of rhythmically repeated linear motion in the horizontal plane *Brit J Psychol* 53 439
- Young L R & Meury J L 1968 A revised dynamic otolith model *Aerospace Med* 39 606
- Young L R, Meury J L & Li Y T 1966 Control engineering approaches to human dynamic space orientation *National Academy of Science Workshop on Orientation in the Exploration of Space NASA SP 115 p 717*

G Melvill Jones M D  
Aviation Medical Research Unit  
Dept of Physiology  
McGill University  
Montreal PQ  
Canada

## LABYRINTHINE AND SOMATOSENSORY CONVERGENCE UPON VESTIBULO OCULAR UNITS

A M Rubin, S C R Liedgren, L M Ödkvist, A C Milne and J M Fredrickson

*From the Laboratory of Otoneurophysiology Department of Otolaryngology University of Toronto Canada  
and the Department of Otolaryngology University Hospital Linköping Sweden*

(Received February 22 1977)

**Abstract** The vestibular nuclei were investigated in 18 adult cats. Vestibulo-oculo-motor neurons were identified by antidromic stimulation of the ascending medial longitudinal fasciculus (MLF). The neurons were subjected to various stimuli: vestibular, neck, forelimb and hindlimb nerve stimulation on both sides. The recording was extracellular with micropipettes containing Fast Green. Only the medial and the superior vestibular nuclei were found to project to the MLF. All projecting units had input from the labyrinths. Excitatory response latencies to ipsilateral labyrinth stimulation never exceeded 3 msec. Both excitatory and inhibitory response latencies could be distributed into different categories. The majority of the neurons did not receive a somatosensory input and surprisingly few convergent units could be seen. Peripheral sensory information apparently plays a minor role in vestibulo-ocular relations.

The labyrinth, vestibular nuclei (VNC) and secondary vestibular fibres to the nuclei of the extraocular muscles via the medial longitudinal fasciculus (MLF) play a major role in the control of conjugate eye movements.

The eye movement control system moves the eye in order to maintain the image of the fixated object on the retina. This image is displaced by the motion of the visual target and by changes in head position, e.g. by rotation (Meiry, 1971). The vestibulo-ocular reflex stabilizes the position by generating compensatory eye movements to counteract movements of the head. Bizzi et al. (1972) have shown that this stabilization of the eye

during head rotation is strongly influenced by various feed back circuits. Peripheral feedback would serve two important purposes. It would compensate for output disturbances, that is, act as a regulator, e.g. for posture adjustments. Secondly, it would act as a sensory mechanism, e.g. for compensatory eye movements. Both of these functions are utilized in the control of movement and posture (Hick & Henneman, 1974), in equilibrium control and visual stabilization. Thus, as a consequence of a head movement, labyrinth receptors and neck proprioceptors are activated and with the assistance of visual information, the reflex loops induce a compensatory eye movement which permits the fovea to remain fixed in relation to a point in visual space.

However, Teixeira & Lackner (1976) have observed that a change in the perceived position of the head in the absence of a physical deviation can influence eye posture. This indicates that, under certain conditions, the influence of neck proprioceptor afferents is not directly reflexive but must be centrally interpreted before effecting oculo-motor commands. Evidence hinting that the area responsible for integrating the vestibular, proprioceptive and visual information is the VNC, has recently been provided by Miles (1974), who found some VNC unitary activity to be correlated with eye movements in certain situations. These units may be involved in the programming of head movements (Zierau et al., 1977).

This project was supported by the Canadian Medical Research Council (grant MA3311) and by the Medical Research Council (grant 14X 4503).

This study was carried out to examine whether or not somatosensory input affects vestibulo-ocular units and if so to investigate the patterns of this input.

## MATERIALS AND METHODS

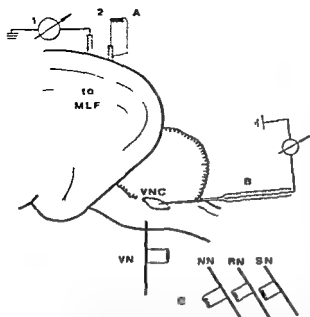
The series included 18 cats weighing 2–3.5 kg. Surgery was performed under halothane/ $I_2O/O_2$  anaesthesia. Tracheostomy was done routinely. Endtidal  $CO_2$  was monitored with a Beckman gas analyser and kept at 3.5–4.5%. A heating pad maintained body temperature at 36–37°C. The anaesthesia was discontinued prior to the recording and the animal was given flumazenil triethiodide (Flaxedil) and artificial ventilation was started. Wounds and pressure points were repeatedly infiltrated with Xylocaine and precautions were taken to ensure that the animal experienced no pain. Throughout the experiments there were no signs of piloerection or dilation of the pupils. After the preparation the animal was placed in a David Kopf stereotaxic frame. The body was supported by a sling such that the limbs hung free. The electrode arrangement is illustrated by Fig. 1. Stimulating bipolar silver electrodes (Teflon coated except for the tip, 125  $\mu$ m in uncoated diameter) were placed on the utricular lateral canal nerve on both sides via a ventrolateral approach to the inner ear. They were kept in place by low melting point (42°C) paraffin and fixed to the skull bone with acrylic cement. Current spread to cochlear and facial nerves was controlled by using intensities less than 3–5 times threshold (for evoked eye deviation) for vestibular nerve (VN) stimulation (Rubin et al., 1977).

The radial nerves (RN) were dissected in both forelimbs and placed on plastic rails containing bipolar stimulating stainless steel electrodes. The same procedure was repeated bilaterally for the sciatic nerves (SN) and the nerves to the cleidotrapezoid muscles (NN). Through a craniotomy exposing relevant brain areas electrodes were stereotaxically placed on the ipsilateral (with respect to the

recording electrode) and contralateral medial longitudinal fasciculi (MLF). The electrode consisted of two insulated stainless steel wires (100  $\mu$ m uncoated diameter, tapered tips 0.5 mm apart) in a glass cylinder with an external diameter of 0.6 mm. These electrodes were used for antidromic activation of VNC units. Correct electrode positioning was determined on the basis of vestibular evoked potentials. During repetitive stimulation of the ipsilateral VN the electrode was slowly lowered towards the MLF. The point at which the largest field potential could be recorded was taken as the location of the MLF. The average response latency was  $1.7(\pm 0.2)$  msec. After the recording current was passed through the MLF electrode to produce a lesion for histological localization. Of course, the method employed does not provide proof of a selective stimulation of the MLF. It is also likely that adjacent structures have been activated.

Glass microelectrodes (2–4 M $\Omega$ , 2 M KCl) saturated with Fast Green FCF (Thomas & Wilson, 1965) were used for extracellular recording in the VNC. It was reached through a posterior cranio-laminectomy and the electrode was angled so as to leave the cerebellum intact. The correct electrode position in the VNC was achieved through stereotaxic calculations and the method described by Shumazu & Precht (1965). At various electrode positions dye was deposited electrophoretically to aid in the subsequent histological analysis. Mechanical stability of the recording system was obtained by covering the exposed area with 3% agar gel.

The microelectrode was connected to an a.c. amplification system. A PDP8E computer was employed for on line analysis of the recordings which also were stored on a Philips analog 7 tape recorder for off line analysis (Sigma V Computer). The microelectrode was lowered through the VNC while stimulating the ipsi and contralateral MLF respectively. Neurons antidromically activated by MLF stimulation were regarded as projecting to the oculomotor nuclei. Criteria for judging the ex-



**Fig 1** Arrangements of stimulating and recording electrodes. **A** is the bipolar electrode which was lowered into the medial longitudinal fasciculus (MLF). A correct position was determined on basis of recorded evoked potentials using a peripheral vestibular stimulus (**1**). This electrode (**2**) was employed to antidromically activate neurons in the vestibular nuclear complex (**VNC**). Extra cellular recording in the VNC took place (**B**). Peripheral stimulation consisted of single electric shocks administered through implanted bipolar electrodes in the vestibular nerve (**VN**), the radial nerve (**RN**), the sciatic nerve (**SN**) and the nerves to the cleidotrapezoid muscle (**SN**) laterally.

citation of a neuron as antidromic have been reviewed by Kelly et al (1975) and the specific problem of the collision test has been further analysed by Fuller & Schlag (1976). The interpretation of the antidromic response is in accordance with their opinions and results. Once a MLF projecting VNC unit was identified its spontaneous activity, if any, was recorded. It was then subjected to various stimuli, bilateral VN stimulation was followed by bilateral RN, SN and NN stimulation.

Peripheral nerve stimulus consisted of square waves (0.1 msec duration frequency 4–1 Hz) and the stimulus intensity was kept at 1.5 times threshold for evoking a muscle twitch prior to immobilization. The evoked neuronal activity was amplified at a band width of 1–3000 Hz to show field potential and unitary activity on the oscilloscope for photo-

graphy. Low frequency was cut off at 300 Hz for triggering standard pulses by spikes to be displayed in post stimulus time histograms (PSTH). Bin widths could be varied for 11 bins per histogram.

The animals were killed with a lethal dose of pentobarbital and perfused with 10% formaldehyde in saline. Serial 50  $\mu$ m thick frozen sections of the brain stem were cut in the plane of the electrode series and stained according to Kluver Barrera. Tracing of electrode sites in the MLF was done on 100  $\mu$ m cresyl violet stained frozen sections. The marks and the lesions aided in identifying recording positions. The boundaries used to separate the individual vestibular nuclei were those described by Brodal & Pompeiano (1957) and Berman (1968).

## RESULTS

97 VNC neurons projecting to the ascending MLF (MLF units) were encountered. Although all four major vestibular nuclei were extensively investigated, MLF units were found to be confined to the superior and medial nuclei (SVN and MVN). The lateral and the descending vestibular nuclei thus contained no cells with antidromic responses to MLF stimulation. This observation is based on an analysis of 344 neurons.

25% of the projecting neurons were found in the SVN and the remaining 75% in the

**Table 1** Types of labyrinthine input for 5 VNC neurons projecting to the MLF

Figures indicate number of neurons

Stimulus	Response in MVN		Response in SVN	
	excitatory	inhibitory	excitatory	inhibitory
Ipsilateral VN	57	15	17	11
Contralateral VN	0	0	0	3
Ipsilateral and contralateral VN	16	5	9	4
Total	73	29	21	18

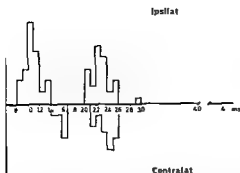


Fig. 2. Response latencies for neurons in the medial vestibular nucleus. All responses are excitatory. Stimulus is as in the following figures: single electric shocks to the VN and contralaterally.

VN. As more tracks passed through the more easily accessible MVN, positive conclusions cannot be drawn regarding the percentage of projecting cells in different nuclei.

#### Labyrinth input to MLF projecting MVN neurons

11 units in the MVN projecting rostrally responded to VN electrical stimulation with an excitation and the majority (78%,  $n=73$ ) were influenced solely by ipsilateral stimulus (Table I). The remaining 22% could be activated from both labyrinths. No neuron was found to increase its spontaneous activity when only a contralateral stimulus was given. Around one third of the 73 MVN neurons were also inhibited (Table I), many by contralateral labyrinthine input. Fig. 2 illustrates the response latencies of the analysed cells when VN stimuli were given. Those units having an increased firing rate due to ipsilateral labyrinth stimulation fell into two latency categories. One group responded monosynaptically (0.8–1.4 msec). The second one also showed a rapid activation (2.0–2.6 msec) possibly including both disynaptic responses. At this latency range the demarcation between late disynaptic and early trisynaptic responses becomes quite indistinct.

Contralateral labyrinth inflow also caused two latency groups (Fig. 2). No monosynaptic responses were observed although both

groups had short latencies. The first one was definitely within the disynaptic range (1.4–1.7 msec) and the second one closely paralleled the corresponding group in the ipsilateral population (2.1–2.6 msec).

The latencies for MVN neurons responding with an inhibition are illustrated in Fig. 3. Due to the difficulty in determining onset of the inhibition, larger bin widths were employed than was the case for excitatory responses (0.5 vs 0.1 msec bins). The number of units in this sample is quite small (29) but it is still evident that ipsilateral VN stimulation gives rise to two distinct short latency groups (less than 10 msec). One population had a rapid inhibitory response, 2.5–4.0 msec indicating that although monosynaptic inhibition was absent, nevertheless few interneurons were involved in this inhibitory pathway. A more complex neuronal system is indicated by the latency of the second population 7–8.5 msec.

Even fewer units (9) were found which responded to contralateral labyrinth stimulus with inhibition. However, on a first approximation it appears as if there are two latency populations which more or less correspond to the ones seen for ipsilateral input.

#### Labyrinth input to MLF projecting SVN neurons

Of the 24 neurons recorded in the SVN, 21 were excited by VN stimulation and 15 of these were also inhibited. The remaining 3 cells showed only an inhibitory response (Table I).

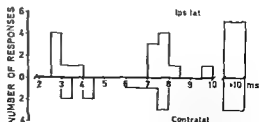


Fig. 3. Inhibitory response latencies for neurons in the medial vestibular nucleus. Ipsilateral and contralateral refer to ipsilateral and contralateral electrical VN stimulation.



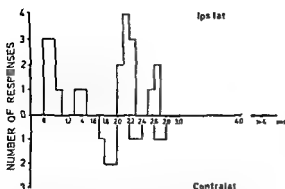


Fig 4 Excitatory response latencies for neurons in the superior vestibular nucleus Ipsilat and contralat refer to ipsi and contralateral electrical VN stimulation

As in MVN the majority (57%,  $n=21$ ) of the excited units could only be activated from the ipsilateral labyrinth. Again, no neurons were observed to be excited from the contralateral labyrinth. Twice as many units in the SVN responded to bilateral labyrinth input as was the case for MVN. The majority of the inhibited neurons (61%,  $n=18$ ) were affected by ipsilateral labyrinth stimulus. Approximately as many cells responded to contralateral as to bilateral stimulation.

SVN units were also analysed in terms of their excitatory response latencies which are given in Fig 4. There is still the problem of the smallness of the sample, which allows more for suppositions than for statements. With regard to ipsilateral inflow, one population demonstrates monosynaptic excitation, 0.8–1.5 msec, while the second one has late disynaptic and/or early trisynaptic response latencies, i.e. the same two groups as were observed in the MVN. Contralateral VN stimulation evoked response latencies in the range 1.7–2.2 msec. Thus the latency grouping of excitable neurons appears to be quite similar for MVN and SVN.

Proportionately more units in the SVN than in the MVN were inhibited (Table 1). Response latencies are illustrated in Fig 5. Although two distinct latency populations were observed when an ipsilateral stimulus was employed, we may still be dealing with a single one, considering the number of cells

Units with response latencies slower than 1 msec were not analysed in terms of latency population grouping. Contralateral labyrinth inflow gave rise to a similar latency pattern was observed for the ipsilateral group (Fig 4). The number of cells was smaller.

#### *Somatosensory input to MLF projecting units in the VNC*

Twenty five neurons in the MVN and 9 in the SVN could be influenced by somatosensory volleys, not only evoked by electrical single shocks to the dissected peripheral nerves but also due to manual stimulation of different body parts, i.e. joint manipulation, touch of fur and skin and "deep" pressure. To 35% of the investigated neurons ( $n=9$ ) demonstrated a convergence of labyrinthine and somatosensory input. No specific pattern with regard to side of labyrinthine activation of the vestibular nucleus, or somatosensory receptive fields could be delineated with certainty, but certain combinations of stimuli seemed to predominate. The MVN cells with ipsilateral vestibular input were in 50% influenced by neck stimuli ( $n=18$ ). None was encountered responding to a neck and hindlimb or forelimb and hindlimb stimulus combination. At least 10 cells were activated but some were inhibited by the somatosensory stimulus as shown in Fig 6. This neuron is located in the MVN. Ipsilateral VN stimulation causes a positive

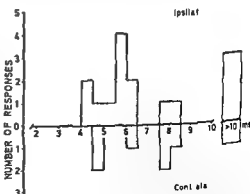
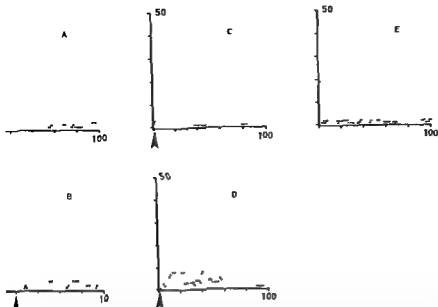


Fig 5 Inhibitory response latencies for neurons in the superior vestibular nucleus Ipsilat and contralat refer to ipsi and contralateral electrical VN stimulation



6 Histograms illustrating the different responses of a neuron in the MVN to vestibular and somatosensory stimuli. Number of spikes on the Y axis and time in msec on the X axis. Arrows indicate onset of stimulus. A and B show the post-excitatory inhibition to ipsilateral VN

stimulus with different time scales 100 and 10 msec respectively (C) Inhibition due to ipsilateral NN stimulus (D) Excitation on contralateral RN stimulation E shows the spontaneous firing rate of the neuron

stimulatory inhibition, while ipsilateral NN stimulation inhibits the spontaneous rate. The latency is around 7 msec. The observed excitation from the neck was usually shorter, 4 msec. When the ipsilateral RN was stimulated the result was an excitation with bursts, the rapid one having a latency of 4 msec.

The somatosensory input to the seven MVN neurons with bilateral vestibular inflow seemed to arise from wide receptive fields in the neck and hindlimb regions bilaterally. Neurons in the SVN responded to somatosensory stimulation in a similar manner as described for the MVN. Receptive fields were frequently located in neck and forelimb regions and activation from hindlimb areas was rarely seen when also neck and/or forelimb fields were involved. The SVN neuron in Fig. 6 has no proven input from hindlimb but from neck and forelimb somatosensory receptors. A unilateral labyrinthine stimulus produces a synaptic activation followed by an inhibitory response and the responses evoked from the con-

tralateral side show roughly the same characteristics.

## DISCUSSION

MLF projecting units were restricted to the SVN and MVN. This is in agreement with the anatomical findings of Tarlov (1972) who was unable to demonstrate direct vestibulo-oculomotor fibres arising in the lateral or descending vestibular nuclei. McMasters et al. (1966) claim to have found vestibulo-oculomotor projection from all four vestibular nuclei which, however, is believed by Tarlov (1972) to be due to incidental damage to the MVN. Studies by Stein & Carpenter (1967) and Gacek (1969) have corroborated the concept of MLF fibres arising only in the SVN and MVN.

In the present study, it would appear that a larger proportion of SVN units projects to the MLF than in the MVN, when proper regard is taken to number of cells recorded and electrode tracks in the two nuclei. This is not

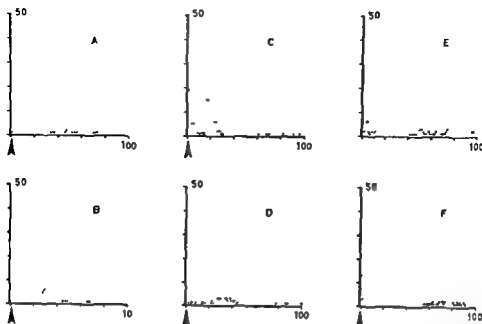


Fig. 7. Histograms illustrating the different responses of a neuron in the SVN to vestibular and somatosensory stimuli. Number of spikes on the Y axis and time in msec on the X axis. Arrows indicate onset of stimulus. A and B show the disynaptic excitation followed by an inhibition elicited from the ipsilateral labyrinth. Time scales are different. (C) Ipsilateral RN stimulus caused this ex-

citatory-inhibitory response while contralateral RN, contralateral NN or SN stimulation had no effect. (D) Response to ipsilateral NN stimulus. (E) Spontaneous firing rate of the neuron. (F) shows the response to contralateral VN stimulus. It has about the same characteristics as in A and B but the latency is about 1 msec longer.

totally unexpected, since many MVN units also project to the cord and the cerebellum, whereas the SVN projection is predominantly directed towards the oculomotor complex (Brodal, 1972).

In the MVN, all MLF projecting units received an ipsilateral labyrinth input, while in the SVN 92% were of this type. Furthermore, no unit could be excited from the ipsilateral labyrinth with a response latency shorter than 3 msec.

This is logical, since we are dealing with a system, often trisynaptic (Szentágothai, 1964), which upon changes in head position must make almost instant compensatory eye movements in order to keep the fixated image stabilized on the retina.

As seen for other projection systems (Rubin et al., 1977) inhibitory input to VNC units was present—though of considerably longer latency than excitatory input. Both excitatory and inhibitory inflow seemed to be split into several latency groups. Such a finding implies

the existence of several different input pathways. Besides the monosynaptic ipsilateral labyrinth input to VNC neurons projecting to the MLF, there are disynaptic and polysynaptic pathways. In addition to the three neuron pathway with a synapse in the VNC, there are multisynaptic pathways which also connect the VNC to the eye muscles (Szentágothai, 1964). Their importance is shown in the experiments by Shanzer (1964).

The MLF projecting VNC units were found to be scarce in somatosensory input and consequently few showed labyrinth-somatosensory convergence. Wilson et al. (1965) observed only 32% of the MLF units to be facilitated by shocks strong enough to activate group III fibres.

Fredrickson et al. (1966) described labyrinthine and somatosensory convergence for a large proportion of analysed VNC neurons. The somatosensory input was predominant in the form of proprioceptive information from the neck and limbs, especially from proprio-

ints According to Pompeiano & Barnes 1971) it is highly improbable that Ia muscle afferents contribute position information direct to the VNC. The present study only deals with the VNC cells projecting to the MLF and here somatosensory and labyrinthine convergence appears to be proportionately less extensive than for the VNC cell population as a whole. However, when this convergence is observed there was a strong preference for input from receptive fields in the upper parts of the body, especially in the neck region. Manual stimulation of the limbs indicated that joint manipulation, above all proximal joints like hips, shoulders and elbows, was frequently the most effective stimulus with regard to somatosensory activation of the neurons. The majority of neurons received antagonistic input from the two sensory systems which is in line with earlier reports (Fredrickson et al., 1966).

Various possibilities may account for the large somatosensory input reported, including methodological factors. However, when an individual semicircular canal is stimulated, eye movements are induced in the planes parallel to the plane of that canal—regardless of the position of the eyes and the orbit or of the head and the neck (Cohen et al., 1966). Under these circumstances there is little need for information from receptors in the neck or other parts of the body.

The stimulus was administered to the nerves from the lateral semicircular canal and the utricle. The otolith organs compensate for static head tilt by inducing counterrolling (Dekleyn 1921). The neck receptors do not contribute significantly to this type of eye movement when the head is subjected to a static tilt (Hannen et al., 1966; Cohen et al., 1970). Dynamic compensatory counterrolling, on the other hand, can be larger—hence the need for inflow from neck afferents.

## ZUSAMMENFASSUNG

Die Vestibularkarte von 18 erwachsenen Katzen wurde untersucht. Vestibulo-okulomotorische Neuronen

wurden durch antidromische Stimulation des Fasciculus longitudinalis medialis (MLF) identifiziert. Die Neuronen wurden verschiedenen Stimulus ausgesetzt: vestibulären Hals Vorder und Hinterbein auf beiden Seiten. Die Registrierung erfolgte extrazellulär mit „fast green“ gefüllten Mikropipetten. Nur bei den medialen und superioren Vestibularkernen wurde eine Projektion auf MLF nachgewiesen. Alle projizierenden Neuronen hatten Affferenz von den Labyrinth. Die Latenzen für exzitierende Antworten auf ipsilaterale Reize des ipsilateralen Labyrinthes waren nie länger als 3 msec. Sowohl anregende als auch hemmende Antwortlatenzen konnten in verschiedene Gruppen getrennt werden. Die Mehrzahl der Neuronen erhielt keine somatosensorische Affferenz und in Übereinstimmung damit fanden wir nur wenige konvergente Neuronen. Periphere somatosensorische Information spielt offenbar eine geringe Rolle für die vestibulo-okulären Verbindungen.

## REFERENCES

- Bergman A. L. 1968. The brain stem of the cat. In *A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. The University of Wisconsin Press, Madison, Wisconsin.
- Buzzi E., Kalil R. E., Morasso P. & Tagliasco C. 1972. Central programming and peripheral feedback during eye-head coordination in monkeys. *Bibl. Ophthalmol.* 82: 220.
- Brodal A. 1972. Some features in the anatomical organization of the vestibular nuclear complex in the cat. In *Basic Aspects of Central Vestibular Mechanisms* (ed. A. Brodal & O. Pompeiano). *Progr. Brain Res.* 37.
- Brodal A. & Pompeiano O. 1957. The vestibular nuclei in the cat. *J. Anat.* 91: 438.
- Cohen B., Krejcová M. & Highstein S. 1970. Ocular counterrolling induced by static head tilt in the monkey. *Fed. Proc.* 29: 454.
- Cohen B., Tokumasa K. & Goto K. 1966. Semicircular canal nerve eye and head movements. The effects of changes in initial eye and head position on the plane of eye movements. *Acta Otolaryngol.* (Stockh.) 61: 168.
- H H. 1966. Convergence and interaction of vestibular and deep somatic afferents upon neurons in the vestibular nuclei of the cat. *Acta Otolaryngol.* (Stockh.) 61: 168.
- Fuller J. H. & Schlag J. H. 1976. Determination of antidromic excitation by the collision test: problems of interpretation. *Brain Res.* 112: 283.
- Gacek C. C. 1969. The course and central termination of first order neurons supplying vestibular end organs in the cat. *Acta Otolaryngol.* (Stockh.) Suppl. 254: 1.
- Hannen R. A., Kabrisky M., Replogue C. R., Hartzler V. L. & Roccaforte P. A. 1966. Experimental determination of a position of the human vestibular system response through measurement of eyeball counterroll. *IEEE Trans. Biomed. Engin.* 13: 65.
- Houk J. & Henneman E. 1974. Feedback control of movement and posture. In *Medical Physiology* (ed. V. B. Mountcastle). C. V. Mosby Co., St. Louis.

- Kelly J S, Simmonds M A & Straughan D W 1975 Microelectrode techniques. In *Methods in Brain Research* (ed P B Bradley) pp 333-377. J Wiley & Sons London
- McMasters R E, Weiss A G & Carpenter M B 1966 Vestibular projections to the nuclei of extraocular muscles. *Am J Anat* 118: 163
- Meiry J L 1971 Vestibular and proprioceptive stabilization of eye movements. In *The Control of Eye Movements* (ed P Bach y Rita & C C Collins) Academic Press New York
- Miles F A 1974 Single unit firing patterns in the vestibular nuclei related to voluntary eye movements and passive body rotation in conscious monkeys. *Brain Res* 71: 215
- Pompeiano O & Barnes C D 1971 Effect of sinusoidal muscle stretch on neurons in medial and descending vestibular nuclei. *J Neurophysiol* 34: 725
- Rubin A M, Liedgren S H C, Ödkvist L M, Milne A C & Fredrickson J M 1977 Labyrinthine inputs to the vestibular nuclei of the awake cat. *Acta Otolaryngol* (Stockh). Accepted for publication
- Shanzer S 1964 Effects of semicircular canal stimulation in monkeys with lesions of the median longitudinal fasciculus. *Fed Proc* 23: 414
- Shimazu H & Precht W 1965 Tonic and kinetic responses of cat's vestibular neurons to horizontal angular acceleration. *J Neurophysiol* 28: 991
- Stein B M & Carpenter M B 1967 Central projection of the vestibular ganglia innervating specific parts of the labyrinth in the rhesus monkey. *Am J Anat* 120: 281
- Szentágothai J 1964 Pathways and synaptic patterns connecting vestibular receptors and ocular motor nuclei. In *The Oculomotor System* (ed M J Bender) Hoeber Medical Division Harper & Row New York
- Tarlov E 1972 Anatomy of the two vestibulo-ocular motor projection systems. In *Basic Aspects of Vestibular Mechanisms* (ed A Brodal & O Petreanu) Progr Brain Res 37
- Teixeira R A & Lackner J R 1976 Influence of different head position on optokinetic nystagmus & eye rotation. *Exp Brain Res* 24: 435
- Thomas R C & Wilson V J 1965 Precise location of Renshaw cells with a new marking technique. *Nature* 206: 211
- Wilson V J, Wylie R M & Marco L A 1968 Sensory inputs to cells in the medial vestibular nucleus. *J Neurophysiol* 31: 176
- Zierau H, Schaefer K P & Süss H J 1976 Head coordination in rabbits. Microrecordings from different brain structures. *Pflügers Arch Ges Ph* Suppl Vol 362: R37

Chr Liedgren MD  
Dept of Otolaryngology  
University Hospital  
S-581 85 Linköping  
Sweden

## INHIBITORY AND PROMOTING EFFECTS OF SUBLIMINAL PENDULAR ROTATION ON OPTOKINETIC NYSTAGMUS IN MAN

O Tokunaga

*From the Department of Otorhinolaryngology Faculty of Medicine  
Kyushu University Fukuoka Japan*

(Received March 17, 1977)

**Abstract** The influence of vestibular stimulation by subliminal pendular rotation on the optokinetic nystagmus in normal human subjects is discussed. Cortical OKN with pendular rotation was promoted in three indexes—eye speed, frequency and total amplitude. The subcortical OKN increased markedly when the slow phase of OKN and the compensatory eye movement resulting from the pendular rotation were in the same direction. However, it was inhibited when the two directions were mutually opposed. From the results obtained in the present experiment and in the previous study by the author, it can be included that the vestibular organs' effect on visual function is not only promotive but also inhibitive.

It is a well known fact that there is a common central pathway and an integration between the vestibular system and the optokinetic system. The present experiment was performed using normal human subjects, in order to study the mechanisms between the two systems.

### MATERIALS AND APPARATUS

The subjects were 20 healthy students, aged 20-23 years. Fig. 1 shows a small optokinetic

cylinder, 10 cm in diameter, 10 cm in height, and 2 kg in weight, having 8 black stripes 1 cm in width on its surface. The cylinder was rotated at a constant speed of 200°/sec. A convex lens of 4 D was placed between the eyes and the cylinder to enlarge the visual field. The optokinetic stimulation was applied to the subjects according to the method of Jung (1953), who described the problem of the intention of the subject in the optokinetic nystagmus (OKN) test. Namely, when the subjects gaze attentively with interest at the stripes on the cylinder, the cortical form of OKN is induced, and when looking unattentively at the cylinder, the subcortical form of OKN is induced.

A rotatory chair of Bárány's type was used for the pendular rotation. The chair was rotated manually under conditions of a pendular rotatory angle of 90° for a period of about 6 sec. Under these conditions no nystagmus

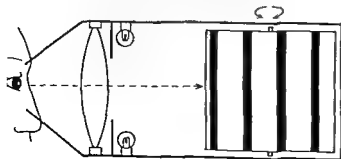


Fig. 1 The optokinetic cylinder

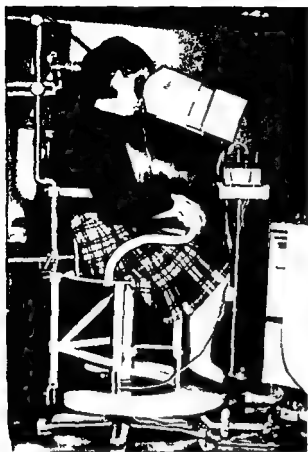


Fig 2 The optokinetic cylinder fixed on the rotatory chair

as observed with eyes closed—or with the eyes open—in the dark.

The subjects were tested sitting in the chair to which was fixed the optokinetic cylinder (Fig 2), in this way they were subjected simultaneously to pendular rotatory stimulation and to optokinetic stimulation. The nystagmographic recording of horizontal eye movements was done using time constants of 2.0 sec and 0.03 sec and a paper speed at 1 cm/sec or 10 cm/sec.

## METHODS AND RESULTS

### Experiment 1

First, 20 subjects were tested by subjecting them to optokinetic stimulation alone. In this experiment, the subjects were ordered to gaze attentively at the movement of the stripes of the cylinder. After an interval of about 10 min the next optokinetic test was done with a subliminal pendular rotatory stimulation. The OKN was recorded for a period of 10 sec at the start of the optokinetic stimulation and its frequency and the total amplitude were calculated, and then the maximum eye speed of the slow phase for the next period of 10 sec. The mean values of R-OKN and L-OKN were used. The absolute values of both the amplitude and the eye speed were calibrated from the values obtained with an eye movement between two visual points  $10^\circ$  apart.

A case of the recorded wave of OKN without and with pendular rotation is shown in Fig 3. The values of OKN obtained are shown in Table I. Table II also demonstrates statistically, by using the table  $t$ , that each value of OKN increased significantly together with the pendular rotation. The increase in eye speed was most remarkable.

### Experiment 2

Five subjects in all were tested in the next study, in order to observe the influence of the subliminal rotatory stimulation on the subcortical OKN. At that time, the subjects were ordered to look unattentively at the cylinder.

As shown in Fig 4 the increase in and the inhibition of OKN were observed in connection with a period of pendular rotation. The wave

Table I Values of cortical OKN without and with pendular rotation

	Without rotation		With rotation	
	mean value	standard deviation	mean value	standard deviation
Frequency	24.8	4.5	29.4	7.0
Total amplitude (mm)	201.0	54.9	233.6	50.6
Max. eye speed of slow phase (deg/sec)	37.4	9.7	54.5	11.0

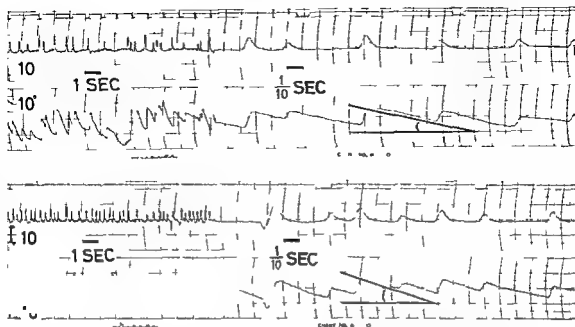


Fig 3 The recorded wave of cortical OKN without (upper) and with pendular rotation (lower). Frequency total amplitude and eye speed of slow phase increased with pendular rotation

R OKN and L OKN form a mirror image. Fig 5 depicts the wave shown in Fig 4. Following the example of R OKN, the OKN was elicited markedly when the slow phase of OKN and the compensatory eye movement in

duced by the rotation from left to right are in the same direction. On the other hand the OKN was inhibited when the two directions were mutually opposed. After the test the subjects mentioned that it was easy to look at the

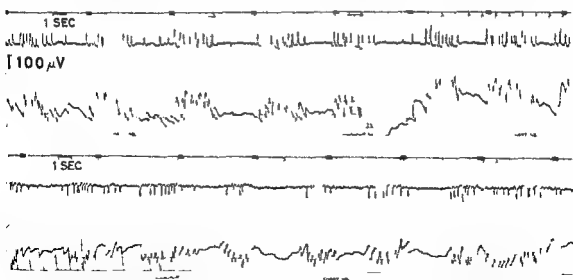


Fig 4 A recorded wave of R OKN (upper) and L OKN (lower) in a subject. These subcortical OKN were alternately promoted or inhibited relating to a period of pendular rotation. R OKN and L OKN form a mirror image. Markers indicate the point where the subject was placed in the left hand position.





Fig 2 The optokinetic cylinder fixed on the rotatory chair

as observed with eyes closed—or with the eyes open—in the dark

The subjects were tested sitting in the chair to which was fixed the optokinetic cylinder (Fig 2) in this way they were subjected simultaneously to pendular rotatory stimulation and to optokinetic stimulation. The nystagmographic recording of horizontal eye movements was done using time constants of 2.0 sec and 0.03 sec and a paper speed at 1 cm/sec or 10 cm/sec.

## METHODS AND RESULTS

### Experiment 1

First, 20 subjects were tested by subjecting them to optokinetic stimulation alone. In this experiment, the subjects were ordered attentively at the movement of the stripes on the cylinder. After an interval of about 10 min the next optokinetic test was done without pendular rotation. Subliminal pendular rotatory stimulation was added. OKN was recorded for a period of 10 sec after the start of the optokinetic stimulation. The frequency and the total amplitude were calculated, and then the maximum eye speed in the slow phase for the next period of 10 sec was calculated. The mean values of R OKN and L OKN were used. The absolute values of both the amplitude and the eye speed were calibrated against the values obtained with an eye movement between two visual points  $10^\circ$  apart.

A case of the recorded wave of OKN without and with pendular rotation is shown in Fig 3. The values of OKN obtained are shown in Table I. Table II also demonstrates statistically, by using the table *t*, that each of OKN increased significantly together with the pendular rotation. The increase in eye speed was most remarkable.

### Experiment 2

Five subjects in all were tested in this study, in order to observe the influence of the subliminal rotatory stimulation on the cortical OKN. At that time the subjects were ordered to look unattentively at the cylinder.

As shown in Fig 4 the increase in and the inhibition of OKN were observed in connection with a period of pendular rotation. The w

Table I Values of cortical OKN without and with pendular rotation

	Without rotation		With rotation	
	mean value	standard deviation	mean value	standard deviation
Frequency	24.8	4.5	29.4	7.0
Total amplitude (mm)	201.0	54.9	233.6	50.6
Max. eye speed of slow phase (deg/sec)	37.4	9.7	54.5	11.0

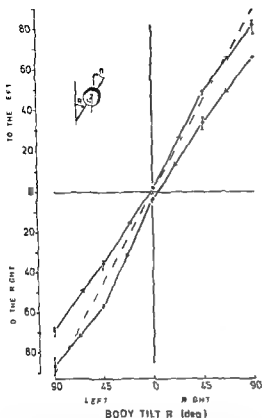


Fig. 1. SV (angle  $\beta$  between adjusted luminous line and median plane of head) as a function of body position ( $R$ ) which was changed by clockwise turning (upper curve) and counterclockwise turning (lower curve). The lines connect the means of the 6-min tests. The vertical arrows indicate the mean readings of the first and last 2 min of each test. The data refer to the average of 15 experiments. 5 subjects were tested three times each. The diagonal interrupted line indicates equality of SV and vertical.

tion has been reported by Clark & Graybiel (1964) for the postural vertical.

The present paper offers a more detailed inspection of these phenomena of the SV.

## METHOD

The apparatus, a biaxial turning bed, and the general procedure were reported in a previous paper (Steinleitner, 1975). In the first series of tests the subjects were turned about a roll-axis (angle  $R$ ) from an upright standing position (pitch angle  $pr_1$ ) through  $270^\circ$  into a left or

right side tilt of  $R 90^\circ$ . This was then the first tested position, for instance,  $R 90^\circ$ -left, which was followed by positions  $R 45^\circ$ -left,  $R 0^\circ$ ,  $R 45^\circ$ -right and  $R 90^\circ$ -right.

Subjects (Ss) were held for 6 min in each position while adjusting a luminous line to the SV 18 times. 5 Ss (20–30 years of age) were tested, each three times.

In a second series, 6 Ss (5 participants from series 1) were investigated in the tilt range beyond side tilt, being turned from  $R 0^\circ$  direct to either  $R 110^\circ$ -right or  $R 160^\circ$ -right, thence to  $R 135^\circ$ -right. During 4 or 6 min in each position they adjusted the line 12 or 18 times respectively. Tests were carried out in two body positions  $pr_1$ , body upright, turning about dorsoventral axis of trunk,  $pr_2$ , trunk prone pitched with respect to head, turning about longitudinal axis of trunk. The head was always turned in the same way, i.e. rolling about naso-occipital axis. In control tests subjects were moved direct from  $R 0^\circ$  to  $R 135^\circ$  and checked there for either 8 and 12 min.

## RESULTS

SV at 5 positions between  $R 90^\circ$ -left and  $R 90^\circ$ -right altered clockwise or counterclockwise.

Fig. 1 and Table I demonstrate significant differences in the perception of SV at positions of the same  $R$  but attained in clockwise or counterclockwise sequence, i.e. reached from the one or the other direction of tilting (hysteresis of SV). At each position, SV readings

Table 1. Statistical evaluation of significance of difference in SV at positions ( $R$ ) attained in clockwise and counterclockwise process.

$T$  test calculation is based on means of SV readings of first 2 minutes.

$R$	$t$	df	$P$
$90^\circ$ left	6.786	4	<0.01
$45^\circ$ left	8.970	4	<0.01
$0^\circ$	8.727	4	<0.01
$45^\circ$ right	22.090	4	<0.01
$90^\circ$ right	3.438	4	<0.05

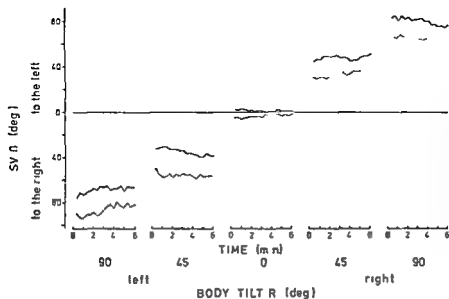


Fig 2 SV as a function of time and body position. Upper and lower curves refer to reaching of position by clockwise and counterclockwise tilt respectively. Same results are used for Fig 1. For further explanation of Fig 1.

deviated from a medium value in a direction opposite to the tilting direction (cf. upper and lower curve of Fig 1).

The differences in SV vary in size with position R, being largest at the side positions (R 90) and smallest at the upright (R 0).

**Decline of hysteresis in time** The differences vary with time too (Fig 2). The distance between the two SV time curves of each position diminishes, due to different slopes of the curves. But although they approach each other, the curves do not reach the same level during

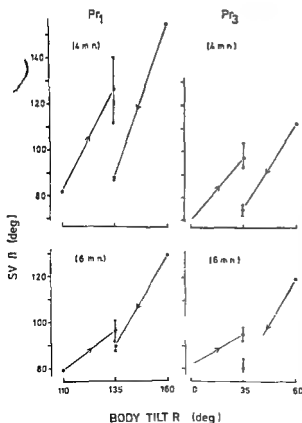


Fig 3 SV as a function of position (R) with body in right or in trunk prone posture (Pr<sub>1</sub>, Pr<sub>3</sub>). R was changed to the final position 135 from preceding positions R 110 and R 160. Vertical arrows indicate first and last time measurements of the 4- and 6-minute experiments (cf. Table 1). Left side: body posture upright, Pr<sub>1</sub>, means of 4-min and 6-min experiments respectively. Right side: body posture trunk prone, Pr<sub>3</sub>, means of 4-min and 6-min experiments respectively. Average of measurements in 2 subjects (upper diagrams) or 4 subjects (lower diagrams) based on three repetitions of each experiment.

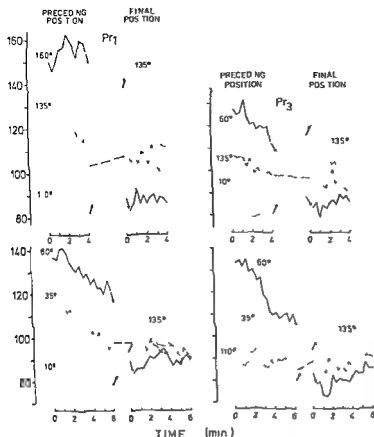


Fig 4  $\bar{V}$  as a function of time and position (R). For further details cf caption to Fig 3. In addition the results of control experiments at R 135 are plotted (lasting 8 and 12 minutes respectively).

he 6 min of test time—not even in the upright position where the intervening distance is smallest. Thus also in this position there is an aftereffect lasting several minutes.

#### *V at positions R 135 right attained from R 110-right and R 160 right*

Again there is a marked difference in SV (Figs 4, Table II). At final position R 135, reached from preceding position R 110 the angle  $\beta$  of  $\bar{V}$  increases steeply, reached from R 160 it drops below these values. The curves of the control tests (i.e. position R 135 tested for 8 and 12 min respectively) are intermediate between the two others. This was expected for the preceding positions where the first 4 (resp. 6) minutes of R 135 fit in between the R 110 and R 160 data. But it holds true also for the final positions. The SV curves of the final 4

(resp. 6) minutes of the R 135 control are in between those succeeding the R 110 and R 160 curves. Both these final curves converge and thus approach the control curve.

Table II Statistical evaluation of difference in SV at positions R 135 right attained from R 110-right and R 160-right

T test calculation is based on the means of the readings of the first 80 sec of the 4 min experiments and of the first 120 sec of the 6-min experiments respectively.

Exp. time	t	df	P
Pr <sub>1</sub>			
4 min	5.968	4	<0.01
6 min	8.265	7	<0.01
Pr <sub>3</sub>			
4 min	4.333	5	<0.01
6 min	4.689	6	<0.01

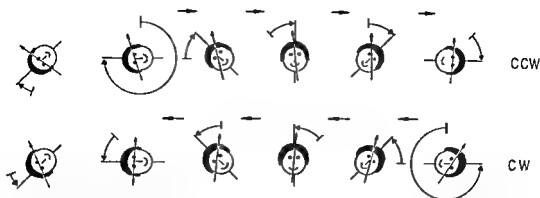


Fig. 5. Illustration of SV perception at various positions (R) as changed in clockwise (upper) and counterclockwise (lower) sequence. Straight arrows in front of head in

indicate SV as perceived in the first minute after reaching position. Curved arrows refer to change from preceding position.

Effect of prone position of trunk is obvious. The curves obtained for body in  $pr_1$  (upright at R0) and  $pr_2$  (trunk prone at R0) differ from each other. In  $pr_1$  the difference in SV between R 110 and R 160 is more pronounced.

## DISCUSSION

The perception of SV is affected by the preceding tilt. In a clockwise sequence of positions, for instance, deviation of the SV is counterclockwise (from a medium value at the upright position). In general terms, the SV deviates in a direction converse to that of position process, which implies perception of the SV as if the body were in a more advanced tilt position of the process than it actually is (Fig. 5). These results correspond to the findings of Clark & Graybiel (1964) on postural after-effects. After spending 2 min (max.) in tilt positions, the subjects were supposed to turn themselves back into the "upright" posture. However, they failed, stopping before reaching the true upright, that is, the posture perceived as upright deviated from the true upright in the direction of the former tilt position, and opposite to the direction of movement. Deviations were more pronounced in labyrinth defective subjects.

A description of our results in terms of A and E phenomena is difficult. Data in the upper curve of Fig. 1, for instance, demonstrate

on the right a tendency towards an E effect, but on the left an A tendency. The opposite tendencies are present in the lower curve. Such denotation can be applied to the deviation in the upright position (R0). This emphasizes that these connotations are of little explanatory value as far as the psychophysiology is concerned.

There is sound evidence linking these findings to the influence of the body's somatosensor system. The hysteresis difference of SV decreases with time, as a result of the different slopes of the two SV time courses involved (Figs. 2, 4). The time courses of SV have been interpreted as a function of adaptation of the somatosensors, interacting with the labyrinth position receptors (cf. introduction). The process of adaptation may be affected by the preceding tilt position. Due to preadaptation of those parts of the somatosensor system already stimulated in the preceding positions, the integrated information from all somatosensors, as fed into perception, may be biased by the newly involved somatosensors. Thus the integrated information indicates a more advanced state of tilt. If, for instance, the subject is turned from a left tilt towards the normal symmetry position, the right hand somatosensors dominate because of the adaptation of the left hand ones, so that the somatosensor system as a whole indicates an advance position not yet

maintained. The subjects in our experiments adjusting the SV as if they were in a more advanced position of the tilt process, in the experiments of Clark & Graybiel subjects stop before reaching the correct upright posture.

The differences in SV (hysteresis) decline continuously for several minutes, due to approximation of the adaptational states of the somatoreceptors. The hypothesis that the somatoreceptors are the main source of after-effects and hysteresis is supported by the findings of Clark & Graybiel, that labyrinthine-defective subjects show a stronger aftereffect than normal ones.

The more pronounced temporal change of V at R135 indicates a stronger influence of somatoreceptors than below R90. This corresponds to the conception of a declining effectiveness of labyrinth position receptors beyond R90 (Schöne & Udo de Haes, 1968, 1971).

Our interpretation of the hysteresis phenomenon is not intended to exclude the possibility of contributions of other factors in generating these aftereffects.

### ACKNOWLEDGEMENT

We want to express our gratitude to Prof. Dr. I. Kohler (Psychol. Inst. Univ. of Innsbruck) for his interest and support of the work. We wish to thank Mrs. Phyllis Rechten for helping in the translation and Renate Alton for her co-operative technical assistance.

### ZUSAMMENFASSUNG

Die subjektive Vertikale (SV) wurde in verschiedenen

Angewinkelungen des Kopfes gegen die Schwerkraft (beginnend mit R90-rechts) eingestellt. Die Stellung R135-rechts wurde von R110-rechts her eingestellt oder von R160-rechts. Die subjektive Vertikale hängt von der vorangehenden Stellung ab (Hysteresis). Bei Stellungswechsel im Uhr

Hysteresisunterschiede in der Wahrnehmung der Stellung und der subjektiven Vertikalen durch adaptive Prozesse im Somatorezeptorensystem bedingt sind, die mit dem Einfluß der labyrintharen Rezeptoren auf diese Vorgänge interferieren.

### REFERENCES

- Clark B. & Graybiel A. 1964 Perception of the postural vertical following prolonged bodily tilt in normals and subjects with labyrinthine defects. *Acta Otolaryngol* (Stockh) 58 143.
- Day I. H. & Wade N. J. 1966 Visual spatial after effect from prolonged head tilt. *Science* 154 1201.
- Fischer M. H. 1927 Messende Untersuchungen über die Gegenrollung der Augen und die Lokalisation der scheinbaren Vertikalen bei seitlicher Neigung des Kopfes des Stammes und des Gesamtkörpers. I. Neigungen bis zu 40°. *v. Graefes Arch. Ophthalmol.* 118 633.
- 1930b Messende Untersuchungen über die Gegenrollung der Augen und die Lokalisation der scheinbaren Vertikalen bei seitlicher Neigung des Kopfes des Stammes. III. Untersuchungen an einem Ertaubten mit Funktionsuntüchtigkeit beider Vestibularapparate und einem einseitig Labyrinthlosen. *v. Graefes Arch. Ophthalmol.* 123 509.
- Guedry F. E. 1974 Psychophysics of vestibular sensation. In *Handbook of Sensory Physiology*, vol. VI/2. Springer, Berlin, New York.
- Ormsby C. C. & Young L. R. 1975 Nonlinear model for the perception of static orientation. *Fortschr. Zool.* 23 288.
- Schöne H. 1967 Über den Einfluß der Schwerkraft auf die Augenrollung und auf die Wahrnehmung der Lage im Raum. *Zeitschr. Vgl. Physiol.* 46 57.
- 1964 On the role of gravity in human spatial orientation. *Aerospace Med.* 35 746.
- Schöne H. & Udo de Haes H. 1968 Perception of the gravity vertical as a function of head and trunk position. *Zeitschr. Vgl. Physiol.* 60 440.
- 1971 Space orientation in humans with special reference to the interaction of vestibular, somesthetic and visual inputs. *Biokybernetik* 111. Materialien 2. International Symposium Biokybernetik. VEB Fischer, Jena. 172-191.
- Steinlechner S. 1977 Interaction of labyrinthine and somatoreceptor input as determinants of the subjective vertical. *Psychol. Research*. In press.
- Wade N. J. 1968 Visual orientation during and after lateral head body and trunk tilt. *Percept. Psychophys.* 3 215.
- 1970 Effect of prolonged tilt on visual orientation. *Quart. J. Exp. Psychol.* 22 423.
- H. Schöne, Dr. phil.  
Max Planck Institut für Verhaltensphysiologie  
D-8131 Seewiesen  
West Germany

## SUCCESSFUL TRANSFER OF ADAPTATION ACQUIRED IN A SLOW ROTATION ROOM TO MOTION ENVIRONMENTS IN NAVY FLIGHT TRAINING

D B Cramer, A Graybiel and W J Oosterveld

*From the Naval Aerospace Medical Research Laboratory, Pensacola FL USA*

(Received January 4 1977)

**Abstract** Two flight students grounded for the reason they were highly susceptible to motion sickness completed their training after gradually adapting to 10 rpm achieved by executing head movements during small step-wise increases in angular velocity. Subject 1 executed a total of about 77 000 head movements within a period of 5 months and Subject 2 executed about 108 000 head movements within a period of 42 days. The transfer of adaptation acquired in the laboratory to most motion environments aloft was good; the notable exception involved weightless maneuvers in the case of Subject 1. Both were on flight status when contacted recently. The opportunity was taken to assess the current motion sickness susceptibility in Subject 1 in the fall of 1975. He reached our (mild) sickness endpoint in the rotating room at 17 rpm; average endpoint is 7-8 rpm. Some practical and theoretical implications are discussed.

If a person riding in a slowly rotating room makes a head movement outside the plane of the platform's rotation, cross coupled accelerations are generated which stimulate the vestibular apparatus in an unusual way. In the stationary state, head movements are associated with a pattern of accelerations that produce vestibular sensations consistent with the visual and proprioceptive sensations asso-

ciated with these head movements past experience. By adding rotation the bular sensations associated with the head movements now include those produced by the cross coupled. This produces a situation where the vestibular sensations are no longer consistent with past visual and proprioceptive sensations; subject's reactions to this unusual state grouped into two classes (Graybiel 1969). The first class consists of reflexive reactions as nystagmus, tumbling or turning sensation and certain visual illusions. The first seems to be a direct response to vestibular stimulation. A second class of response directly related to the vestibular stimulation constitutes the signs and symptoms of motion sickness. Inasmuch as these signs and symptoms have their immediate origins in the vestibular systems, one must postulate a tentative linkage between vestibular and vestibular systems as an important element in the causation of this form of motion sickness (Graybiel, 1969). The signs and symptoms arising from this unusual vestibular stimulation have been well studied and a semi-grading method is available (Graybiel 1968).

It has been shown that subjects who perform sufficient head movements at one time can asymptotically reach the

This research was conducted under the sponsorship of the Bureau of Medicine and Surgery MR 041 01 010120 and the Office of Life Sciences NASA Johnson Space Center Houston Texas. Opinions and conclusions contained in this report are those of the authors and do not necessarily reflect the views or endorsement of the Navy Department.

lar velocities which would otherwise be intolerable (Graybiel & Knepton, 1972). By having the subject execute a schedule of head movements at each increment in angular velocity, one has a simple method of providing adaptation to rotation. This scheme is called the incremental adaptation schedule. If the stimulus level of the incremental adaptation schedule is excessively high, the incidence of motion sickness will force the termination of adaptation. Although the relationship between adaptation and motion sickness is not well understood, it is possible, using sufficiently small increments in rpm to achieve adaptation without overt symptoms of motion sickness.

Subsequent experience with incremental adaptation (Graybiel et al., 1975) has shown that this acquired adaptation has two components. The first to occur is a direction specific adaptation which decays in hours after the cessation of rotation. This direction specific adaptation provides increased tolerance to rotation only in the direction employed in the incremental adaptation schedule. It is also associated with a reduced tolerance to head movements at zero velocity and an even lower tolerance to head movements performed with rotation in the opposite direction. The rather rapid decay of the direction specific adaptation reveals a second component of adaptation which is not direction specific and decays slowly over days. This second component of adaptation is not associated with symptoms at zero velocity and does afford increased tolerance to head movements performed with rotation in the opposite direction. This second

situation the stimulus level is so high that the prompt emergence of air sickness does not permit the occurrence of any significant adaptation. A similar stimulation may be created in the laboratory by repeatedly exposing the subject to a high angular velocity without the benefit of incremental adaptation. To test the practical usefulness of this phenomenon, it was decided to determine whether laboratory conducted incremental adaptation could be beneficial to student aviators with a history of severe air sickness.

## MATERIAL AND METHODS

Subjects for this experiment were two flight students who were dropped from flight training due to repeated episodes of severe air sickness. Both students had a life long history of motion sickness. Other than the unusual history of motion sickness, medical examination revealed two young, healthy adult males, both highly motivated to remain in the flight program. By history and on the basis of their previous performance in the flight program, these two students demonstrated an incidence of air sickness far above average and one would expect a high susceptibility to motion sickness. This suspicion was confirmed in both students where comprehensive vestibular testing revealed normal function with the exception of a very high susceptibility to motion sickness.

The rotating device used in this experiment is the Slow Rotation Room I (SRR) at the Naval Aerospace Medical Research Laboratory in Pensacola, Florida. The experiment is conducted inside a windowless, air conditioned, circular room which is 10 feet in diameter and 7 feet high. This room is attached to a large, high mass centrifuge that is capable of very smooth rotation at angular velocities from one to thirty revolutions per minute (Hixon, 1963).

By means of controlled vestibular stimulation each subject is well adapted to each increment of angular velocity. The rotation is provided by the SRR rotating at constant angular

aviators are dropped from flight training because of repeated episodes of severe air sickness. It is reasonable to assume that in their



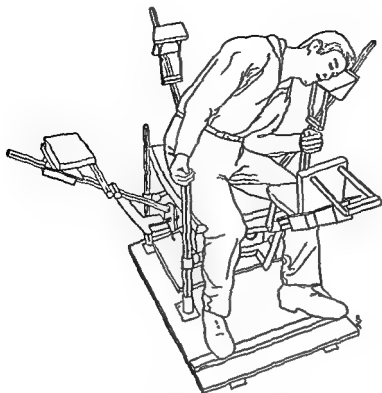


Fig 1

velocities. The vestibular stimulation consists of paced head movements. In this procedure the subject sits upright and upon command from a tape recorder he makes head move-

to the left, right, forward and back.

As shown in Fig 1, the angular dis-

of the head movement is controlled by the placement of pads in each direction of the head movement at an angle of 45 degrees from the vertical. The subject moves his head in the desired direction until he lightly touches the appropriate pad. The commands from the tape recorder specify a given direction every four seconds with the command to return to the upright following the initial command by 2 sec. With this arrangement a discrete head movement is made every 2 sec and at the end of 480 such head movements (16 minutes) the subject is given a three to five minute rest period during which he sits quietly with his head in the upright position. The incremental adaptation schedule will be designed on a daily basis by the authors with the objective of keeping the stress level as high as possible yet avoiding significant motion sickness. Meas-

urements of the tumbling ('giant hand illusion' will be made at each new increment of angular velocity to estimate the intensity of the vestibular stimulation. The severity of motion sickness will be measured continuously using a previously described grading system (Graybiel et al, 1968), which is summarized in Table I. At the end of each daily rotation movements will be immediately conducted at zero velocity to assess the level of acquired direction specific adaptation. It has been proposed that the acquired adaptation will be not direction specific is continually overtaken yet always lagging behind the acquisition of direction specific adaptation (Graybiel et al, 1975). If a subject performs sufficient head movements at a given angular velocity he then continues his head movements at that velocity without any incidence of motion sickness. This occurs, presumably, because he has continued his adaptation to the rotating environment for a period of time long enough to permit the decay of the more transient motion specific adaptation to occur, even while rotating. If the stress level is properly

Table I *Scoring severity of acute motion sickness*

Category	16 points	8 points	4 points	2 points	1 point
nausea syndrome	Vomiting or retching	Nausea* II III	Nausea I	Epigastric discomfort	Epigastric awareness
in color		Pallor III	Pallor II	Pallor I	Flushing
old sweating		III	II	I	
creased salivation		III	II	I	
owness		III	II	I	
in					Headache
entral nervous system					Dizziness
					Eyes closed $\geq$ II
					Eyes open III

III=severe or marked II=moderate I=slight

sted, the subject will display minimal illusions at each new increment in angular velocity and will transiently develop no more than III or two motion sickness points throughout station

At the conclusion of rotation the subjects could remain essentially asymptomatic during the head movements at zero velocity. If the stress level is excessive the illusions will be more prominent, the motion sickness more severe, and the post-run head movements will elicit frank motion sickness. Each subject began at one rpm and worked his way to 10 rpm as quickly as possible.

## RESULTS

The first attempt at adaptation was conducted with Subject 1, whose motion sickness susceptibility was somewhat lower than that of Subject 2. These results are displayed in Table II. This first experiment was conducted over a 7 day period and involved rotation on 6 days. Rotation was always in the counter-clockwise (CCW) direction.

On the first day Subject 1 reached 5 rpm and experienced no motion sickness throughout the day. The subject executed a total of 4200 discrete head movements. This corresponds to exactly 4.0 hours making head movements and was accomplished in 6.18 hours of rotation. Illusions were not prominent at each new increment in angular velocity. Due to a technical oversight, no post-run head

movements were performed on his first day.

On the second day the subject reached 6 rpm and developed no more than 1 motion sickness point while rotating. He performed a total of 5760 head movements (3.2 hours) in 6.85 hours of rotation. Illusions were clearly present at first reaching 5 and 6 rpm. After stopping the subject developed 5 motion sickness points in 115 head movements.

On the third day the subject reached 8 rpm and displayed no more than 2 motion sickness points but remained at 1 point throughout most of the day. He performed 6240 head movements (3.47 hours) in 7.30 hours of rotation. Illusions were present but not prominent. During the post-run head movements the subject developed 6 motion sickness points in 90 head movements.

On the fourth day the subject did not exceed 8 rpm. He developed a maximum of 3 motion sickness points and displayed 2 points for much of the day. He performed 4320 head movements (2.4 hours) in 7.0 hours of rotation. Illusions were present but not prominent at 6-8 rpm. Upon stopping the subject developed 4 motion sickness points in 120 head movements.

On the fifth day the subject reached 10 rpm. He displayed a maximum of 2 motion sickness points at any time during rotation and was asymptomatic at 10 rpm. The subject executed a total of 6720 head movements (3.73 hours) in 8.05 hours of rotation. Illusions were detectable but not prominent. Upon stopping

Table II Incremental adaptation, Subject 1

Date	Run day	Angular Velocity (rpm)										Daily total	Average MS score	Post run HVI	For MS S.c.
		0	1	2	3	4	5	6	7	8	9	10			
8 Jan '71	1 (CCW)	0.96	0.96	1.92	1.92	1.44							7.20	0	
9	2					1.92	3.84						5.76	1	0.12
10	3					0.48	0.96	3.84	0.96				6.24	1	0.09
11	4						0.48	0.96	2.88				4.32	2	0.12
13	5							0.48	0.48	0.48	3.84	1.44	6.72	1	0.12
14	6										0.96	2.40	3.36	1	0.12
		0.96	0.96	1.92	1.92	3.84	5.76	5.28	4.32	4.80	3.84				0.57
		Grand total 34.17													
6 May '71	1 (CCW)	0.96	0.96	1.92									3.84	0	0.24
7	2	0.12	0.12	0.12	2.88								3.24	1	0.24
10	3	0.12	0.12	0.12	0.48	1.92							2.76	0	0.24
11	4	0.12	0.12	0.12	0.12	0.48	1.80						2.76	1	0.24
12	5	0.12	0.12	0.12	0.12	0.12	0.48	1.92	1.92				4.92	2	0.24
13	6	0.12	0.12	0.12	0.12	0.12	0.12	0.12	1.92				2.76	0	0.24
14	7	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.48	1.92			3.24	1	0.24
17	8	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.96	3.84		5.76	1	0.24
		1.80	1.80	2.76	3.96	2.88	2.64	2.28	4.44	2.88	3.84				1.92
		Grand total 31.20													
13 June '71	1 (CW)	0.96	0.96	0.96	0.96	0.96	0.96						5.76	1	0.24
2	2	0.12	0.12	0.12	0.24	0.24	0.48	0.96	0.96	0.96	0.96		5.16	1	0.24
		1.08	1.08	1.08	1.20	1.20	1.44	0.96	0.96	0.96	0.96				0.48
		Grand total 11.40													

\* 1.00 = 1000 individual head movements spaced at two second intervals

the subject developed only 2 motion sickness points in 120 head movements

On the sixth day the subject spent most of time at 10 rpm. He displayed a maximum of 2 motion sickness points and was asymptotic by the end of the day. The subject executed 3360 head movements (1.87 hours) in 4.57 hours of rotation. Illusions were present but not prominent. Upon cessation of rotation the subject developed 3 motion sickness points in 120 head movements.

On the seventh and eighth days the subject was flown in an aircraft especially prepared for studying air sickness. The subject displayed normal susceptibility which was interpreted as a significant improvement in his condition. Of the various maneuvers employed, the subject was most sensitive to 'porpoising' which involved a few seconds of weightlessness. Several days later he participated in studies involving zero g parabolas of 30-45 sec duration. Here he displayed such high air sickness susceptibility as to indicate that the in-

cremental adaptation had afforded little protection for this particular type of maneuver.

Following the first incremental adaptation periodic measurements of motion sickness susceptibility were made to estimate the rate of decay of the acquired adaptation. All these tests were performed in the CCW direction, the same as that of the first incremental adaptation. At 12 days after the completion of the first study, there was minimal waning of the acquired adaptation. At 33 days there was significant waning and at 58 days the subject had returned to his previous baseline susceptibility.

Dissatisfaction with the incidence of motion sickness in the first incremental adaptation led to the decision to attempt a second similar experiment. The objective was to examine the effects of lowering the stress level of the incremental adaptation schedule so as to reduce the incidence of illusions and motion sickness while rotating. This in turn would hopefully reduce the incidence of motion sickness.

Table II Incremental adaptation, Subject 2

Date	Run day	Angular Velocity (rpm)										Daily total	Average MS score	Post run HM	Post run MS score
		0	1	2	3	4	5	6	7	8	9	10			
Mar '71	1 (CCW)	1 92 <sup>a</sup>	1 44	1 92	3 84							9 12	3	0 24	12
Apr '71	2	1 92	3 84	1 92								7 68	2	0 24	6
	3	1 92	2 40									4 32	1		
	4a	1 92	3 84									5 76	0	0 24	0
	5a		2 88	0 96								3 84	0	0 24	0
	6a		1 92	1 92								3 84	0	0 24	0
	7b		0 96	2 88								3 84	0	0 24	2
	8b			2 88	0 96							3 84	0	0 24	1
	9b			0 96	2 88							3 84	0	0 24	1
	10b				2 88	0 96						3 84	2	0 24	8
	11b				1 92	1 92						3 84	0	0 24	1
	12b				0 96	3 84						4 80	0	0 24	1
	13b					1 92	2 88					4 80	1	0 24	5
	14b						2 88	1 92				4 80	1	0 24	2
	15c						0 48	0 48				0 96	4		
	16b		0 48				0 48	2 88				3 84	2	0 24	7
	17b	0 12	0 12	0 12	0 12	0 12	0 12	1 68	2 88			5 28	2	0 24	11
	18b	0 12	0 12	0 12	0 12	0 12	0 24	0 48	1 20	0 96		3 48	2	0 24	7
May '71	19b	0 12	0 12	0 12	0 12	0 24	0 24	0 72	0 48	1 44		3 60	4	0 24	6
	20b	0 12	0 12	0 12	0 12	0 24	0 24	0 24	0 48	2 16		3 84	3	0 10	10
	21c	0 12	0 12	0 12	0 12	0 24	0 24	0 24	0 48	1 20		2 88	2	0 08	6
	22c	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 36	0 96	2 28	2	0 21	6
	23b	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 36	1 20	2 52	3	0 20	7
	24c		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 24	2 40	3 48	2	0 15	9
	25	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 24	0 72	1 92	1	0 18	7
		8 64	18 84	14 52	14 52	10 56	7 80	9 12	6 00	6 96	5 28			5 00	
													Grand total	107 24	

1 00-1 000 individual head movements spaced at two second intervals

<sup>a</sup> 10 mg d amphetamine sulfate, orally<sup>b</sup> 5 mg d amphetamine sulfate, orally<sup>c</sup> 2 5 mg d amphetamine sulfate orally

caused by the post run head movements. In this design the daily head movements always started at 1 rpm. This second CCW incremental adaptation was started 80 days after the completion of the first.

On the first day Subject 1 reached 3 rpm and was essentially asymptomatic throughout the day. He performed 3 840 head movements (13 hours) in 3 50 hours of rotation. Illusions were not noted. Upon the cessation of rotation, the subject developed only 1 motion sickness point in 240 head movements.

On the second day the subject reached 4 rpm and briefly displayed 2 motion sickness points (1 hour) in 240 head movements. He performed 4 800 head movements (8 hours) in 3 50 hours of rotation. Illusions were not detected. Upon stopping the subject remained

asymptomatic through 240 head movements.

On the third day the subject reached 5 rpm and briefly displayed a single motion sickness point at 1 rpm. He performed 2 760 head movements (1 53 hours) in 2 4 hours of rotation. Illusions were not reported. After stopping, the subject remained asymptomatic during 240 head movements.

On the fourth day the subject reached 6 rpm. He remained essentially asymptomatic but transiently developed 2 motion sickness points after a momentary power failure. The subject executed 2 760 head movements (1 53 hours) in 2 4 hours of rotation. Illusions were not reported. Upon stopping, the subject developed a single motion sickness point in 240 head movements.

On the fifth day the subject reached 8 rpm

He was intermittently symptomatic, displaying one or two points for much of the day. The subject executed 4920 head movements (2.73 hours) in 3.95 hours of rotation. Illusions were not noted. Upon halting, the subject developed a single motion sickness point in 240 head movements.

On the fifth day the subject reached 8 rpm. He was intermittently symptomatic, displaying one or two points for much of the day. The subject executed 4920 head movements (2.73 hours) in 3.95 hours of rotation. Illusions were not noted. Upon halting, the subject developed 2 motion sickness points in 240 head movements.

On the sixth day the subject did not exceed 8 rpm and remained asymptomatic throughout rotation. He performed 2760 head movements (1.53 hours) in 2.5 hours of rotation. Illusions were not noted. Upon stopping, the subject developed 3 motion sickness points in 240 head movements.

On the seventh day the subject reached 9 rpm and intermittently scored 2 motion sickness points on two occasions during the day. He performed 3240 head movements (1.8 hours) in 2.85 hours of rotation. Illusions were not noted. Upon the cessation of rotation the subject displayed 2 motion sickness points in 240 head movements.

On the eighth day, the subject reached 10 rpm and he briefly displayed 2 motion sickness points upon initially reaching 10 rpm. He performed 5760 head movements (3.2 hours) in 4.3 hours of rotation. Illusions were absent. Upon stopping, the subject remained asymptomatic in 240 head movements.

Although the second incremental adaptation employed slightly fewer head movements than the first, the successful adaptation to 10 rpm was accomplished with less motion sickness and a much lower incidence of illusions. Provocative tests to assess motion sickness susceptibility (Kennedy & Graybiel 1965) were conducted at 7 and 8 days after the completion of the second incremental adaptation. When compared with the earlier baselines be-

fore both adaptation experiments there was substantial reduction in motion sickness susceptibility. When this test was conducted in the direction opposite to that of both incremental adaptations, i.e. clockwise there was no evidence of transfer of adaptation to opposite direction. This result was surprising since some transfer was expected. To evaluate this possibility in greater detail, it was decided to conduct a clockwise (CW) incremental adaptation.

Subject 1 started a CW incremental adaptation 14 days after the conclusion of the second CCW adaptation experiment. The technique was to be the same as the second CCW experiment and the goal would be 10 rpm CW.

On the first day the subject reached a surprising 6 rpm and displayed a maximum of 2 motion sickness points during the day. He performed 5760 head movements (3.2 hours) in 4.75 hours of rotation. Illusions were reported upon first reaching 5 rpm. Upon stopping, the subject developed 2 motion sickness points in 240 head movements.

On the second day the subject reached 8 rpm. At one point he briefly developed 3 motion sickness points but was back to 1 point within an hour. He performed 5160 head movements (2.87 hours) in 4.45 hours of rotation. Illusions were not present. Upon stopping, the subject developed 1 motion sickness point in 240 head movements. The subject's rapid progress to 10 rpm CW was most likely due to transferred adaptation from the second CCW experiment.

At this point the subject was returned to flight training but due to a recurrence of chronic sinusitis, he did not immediately return to flying status. Because of some difficulty in controlling this chronic sinusitis, Subject 1 was temporarily suspended from flying. However, the problem finally subsided and the subject finished flight training with little difficulty. He is presently in an operational flying billet and periodic follow-up indicated no abnormal incidence of motion sickness. In the fall of 1975 Subject 1 began

turned to Pensacola and it was possible to measure his motion sickness susceptibility. At this time he displayed at typical (d) endpoint at 17 rpm which is well above average of 7-8 rpm.

The incremental adaptation of Subject 2 consists of a single, lengthy adaptation to 17 rpm CCW. Some difficulty was anticipated that Subject 2 was found to be one of the most motion sickness susceptible individuals ever tested at the Naval Aerospace Medical Research Laboratory. The plan was essentially the same as employed with Subject 1. The results of this experiment are displayed in Table III.

On the first day Subject 2 reached 4 rpm in 15 rpm increments. He was symptomatic almost the entire day, averaging about 3 motion sickness points and once reaching 8 points he executed 9120 head movements (507 turns) in 9.5 hours of rotation. Illusions were always present and prominent above 3.25 rpm. Upon halting, the subject developed 12 motion sickness points in 240 head movements. Although the stress level was intentionally designed to be low, it was still excessive for this highly susceptible subject.

On the second day the subject did not exceed 2.5 rpm. The subject was symptomatic much of the time and averaged 2 motion sickness points. He performed 7680 head movements (427 turns) in 8.7 hours of rotation. Illusions were prominent above 1.75 rpm. Upon halting, the subject developed 6 motion sickness points in 240 head movements. Again the stress level was excessive.

On the third day the subject did not exceed 1.75 rpm. Throughout the day he did not develop more than 1 motion sickness point. He performed 4320 head movements (24 turns) in 3.25 hours of rotation. Illusions were less prominent than on the previous 2 days. Due to technical difficulties, post run head movements were not conducted on this day. In view of the unusually slow progress toward 10 rpm, it was decided to attempt the incremental adaptation with the use of an effective anti-

motion sickness drug, d amphetamine sulfate (Graybiel & Wood, 1969; Wood & Graybiel, 1972).

On the fourth day the subject did not exceed 2 rpm. This run employed 10 mg d amphetamine sulfate p.m. and the subject was asymptomatic throughout the day. He performed 5760 head movements (32 turns) in 5.4 hours of rotation. Illusions were present but not prominent. Upon stopping, the subject remained asymptomatic in 240 head movements. Using this technique the subject gradually worked his way to 7 rpm, reaching it on the sixteenth day. The subject generally averaged one or two motion sickness points, though the trend was toward greater motion sickness at higher angular velocities. He performed approximately 4000-5000 head movements per day. Illusions remained present and were occasionally prominent. Post-run head movements were associated with gradually increasing motion sickness scores, reaching 8 points on the sixteenth day. At this point the drug was combined with the technique of starting all daily head movements at 1 rpm.

From the seventeenth through the twenty-fourth day the subject gradually worked his way to 10 rpm. D amphetamine sulfate and the technique of starting all daily head movements at 1 rpm were continued. The subject was continually symptomatic and averaged about 2 motion sickness points during each day. He performed about 3000 head movements per day. Illusions were almost always present but rarely prominent.

The post run head movements produced from 6-10 motion sickness points.

On the twenty-fifth day the subject again reached 10 rpm and no drug was employed for this run. The subject displayed only 1-2 motion sickness points throughout the day. He performed 1920 head movements (107 turns) in 2.4 hours of rotation. Illusions were present but not prominent at 10 rpm. Upon stopping, the subject developed 7 motion sickness points in 180 head movements.

Subject 2 subsequently returned to flight

training which he completed with no usual difficulty with air sickness. He is presently in an operational flying billet and periodic follow up has not revealed any abnormal incidence of air sickness.

## DISCUSSION

On the basis of the results of this experimental probe and the reports of other investigators (Dowd & Cramer, 1967), it is altogether likely that the incremental adaptation to 10 rpm was beneficial to the two flight students. Firm conclusions are difficult to achieve with such a limited number of subjects.

However, both subjects have long felt that the adaptation experiments were of considerable aid in completing their flight training. It is clear that established laboratory tests demonstrate that these two subjects were able to reduce their motion sickness susceptibility while associated with the Laboratory. The relationship between the reduced motion sickness susceptibility upon leaving the Laboratory and the subsequent success in flight training requires more careful examination. If, for example, the same tests that were used to measure motion sickness susceptibility before and after adaptation could be continued through flight training, then one might gain some insight into the relationship between incremental adaptation and improved flight training performance. To obtain a better comparison, students with comparably high susceptibility might be paired, one receiving incremental adaptation and the other continuing in the flight program. It would also be useful to periodically measure the motion sickness susceptibility of normal students as they progress through training. It is probable that the motion sickness susceptibility of student aviators as a group decreases as they progress through training. This effect must be considered before estimating any improvement attributed to vestibular adaptation.

There are several aspects of the data which deserve additional comment. In the case of

Subject 1, there was good transfer of laboratory acquired adaptation to flight maneuvers with the exception of those involving weightlessness. A possible explanation may lie in the fact that weightlessness exerts a major effect upon the otolith apparatus whereas the vestibular stimuli employed in the laboratory primarily condition the semicircular canals. Since one would not expect a conditioning procedure involving the canals to necessarily transfer to the otolith apparatus, it is then understandable that the incremental adaptation to 10 rpm afforded no protection against weightlessness. After the second CCW incremental adaptation of Subject 1, a motion sickness susceptibility test failed to reveal any significant adaptation to the opposite direction. This is surprising in view of earlier work (Reason & Graybiel, 1969) which predicted substantial transfer. When a subsequent CW incremental adaptation was conducted, the rapidity of subject's progress clearly implied considerable transfer from the previous adaptation in the opposite direction. The only explanation presently available is that this test was conducted prematurely, before the complete disappearance of the direction specific component of adaptation.

In the case of Subject 2, the facilitation of adaptation through the use of drugs represents an interesting possibility that requires further investigation. Whether d-amphetamine in fact promotes adaptability by suppressing motion sickness cannot presently be proven. With Subject 2, the decision to employ a drug was largely based on the desire to continue the incremental adaptation. The initial response to the drug was significant but due to the subject's complaints of nervousness the dosage was gradually lowered. What effect increased dosage would have had on the increased motion sickness symptomatology above 6 rpm is not known.

The practical value of incremental adaptation is that it provides a method of reducing air sickness susceptibility which, although time consuming, can be accomplished safely.

ily, and inexpensively with a minimal investment in equipment. This method does not involve the elicitation of motion sickness. Although the data presented here were collected in a slowly rotating room, there is no reason why the technique could not be arranged to use a simple rotating chair. From a theoretical viewpoint, incremental adaptation represents a flexible experimental technique for gaining insight into the progress of vestibular adaptation. By regulating the rate of rotation, the angular velocity, and the number of head movements, the investigator can reliably generate a variety of vestibular stress levels that range from the subthreshold to those which are frankly provocative of motion sickness. The relationship between motion sickness and adaptation is not well understood. It has been reported that adaptation can occur without incurring significant motion sickness (Graybiel et al., 1968) and this has again been shown in these results. However, very little is known about the circumstances promoting optimal adaptation. From existing data it seems probable that motion sickness is not a necessary element of adaptation. This still allows the possibility that motion sickness actually retards adaptation, which would seem to explain the behaviour of some student aviators. If this could be true then the present method of making decisions on the presence of early or mild motion sickness symptomatology may not prove to be very efficient. In the present design the presence or absence of the tumbling illusion was noted with the initial head movements at each new increment of angular velocity. Although this information can be generally related to the adaptation process it is not presently clear how it alone might be used to establish an incremental adaptation schedule. In summary, two well motivated flight students with a lifelong history of motion sickness were referred to the Laboratory because of persistent air sickness of such severity as to jeopardize their continued participation in the flight program. By executing numerous

paced head movements on a rotating room of gradually increasing velocity, both students substantially reduced their motion sickness susceptibility to the laboratory rotating environment. After developing an essentially asymptomatic tolerance to 10 rpm through the technique of incremental adaptation, they returned to the flight program. Both students completed flight training and are presently in operational flying billets where they have experienced no unusual incidence of motion sickness. In these two cases it was possible to employ a recently described adaptation to vestibular stimuli which permits the effective transfer of reduced motion sickness susceptibility from the laboratory rotating environment to an operational flight situation.

## ZUSAMMENFASSUNG

Zwei Flugschüler, fluguntauglich wegen starker Anfälligkeit für Luftkrankheit, vollendeten ihre Ausbildung nachdem sie sich allmählich an 10 rpm adaptiert hatten. Sie erreichten dies indem sie Kopfbewegungen bei schrittweise vergrößerten Winkelgeschwindigkeiten ausführten. Testperson 1 führte insgesamt etwa 77 000 Kopfbewegungen aus in etwa fünf Monaten und Testperson 2 etwa 108 000 in 42 Tagen. Die Übertragung der im Labor erworbenen Adaptation auf die meisten Bewegungsformen im Flug war gut; die bemerkenswerte Ausnahme betraf Manöver mit Schwerelosigkeit. Im Falle der Testperson 1 Beide waren flugtauglich als sie kurzlich befragt wurden. Die Gelegenheit wurde benutzt die gegenwärtige Anfälligkeit für Luftkrankheit bei Testperson 1 im Herbst 1975 festzustellen. Er erreichte unseren (schwachen) Luftkrankheitsendpunkt in der rotierenden Testkammer bei 17 rpm; der mittlere Endpunkt liegt bei 7-8 rpm. Einige praktische und theoretische Folgerungen sind diskutiert.

## REFERENCES

- Dowd P J & Cramer R L 1967 Habituation transference in conical accelerations *Aerospace Med* 38 1103
- Graybiel A 1969 Structural elements in the concept of " " " " " "
- Graybiel A & Wood C H 1969 Rapid vestibular adaptation in a rotating environment by means of controlled head movements *Aerospace Med* 40 638



- Graybiel A , Wood, C D , Knepton, J M , Hoche, J P & Perkins, G F 1975 Human assay of antimotion sickness drugs *Aviat Space Environ Med* 46 1107
- Graybiel, A , Wood, C D , Miller II, E F & Cramer, D B 1968 Diagnostic criteria for grading the severity of acute motion sickness *Aerospace Med* 39, 453
- Hixon, W C 1963 Instrumentation for the Pensacola Centrifuge-Slow Rotation Room I Facility NSAM-875 NASA R 37, Pensacola, Fla , Naval School of Aviation Medicine
- Kennedy, R B & Graybiel A 1965 The Dial Test A standardized procedure for the experimental production of canal sickness symptomatology in a rotating environment NSAM 930, NASA R 93 Pensacola, Fla , Naval School of Aviation Medicine
- Reason, J T & Graybiel, A 1969 Adaptation to Cor. accelerations its transfer to the opposite direction a function of intervening activity III zero velocity NAMI 1086 Pensacola Fla , Naval Aerospace Medical Institute
- Wood, C D & Graybiel A 1972 Theory of antimotion sickness drug mechanisms *Aerospace Med* 43 76

*D B Cramer, M D*

*Naval Aerospace Medical Research Laboratory  
Pensacola, FL, USA*

## ULTRASONIC IRRADIATION OF THE GUINEA PIG LABYRINTH

### *Correlation of Morphology and Intralabyrinthine Temperature*

P.-G. Lundquist, R. A. Schindler and J. Stahle

*From the Department of Otolaryngology Karolinska sjukhuset Stockholm King Gustav V Research Institute Stockholm and the Department of Otolaryngology Akademiska sjukhuset Uppsala Sweden*

(Received May 20 1977)

**Abstract** The effects of high frequency ultrasound on the inner ear structures after irradiation of the lateral semicircular canal have been analysed by means of light and electronmicroscopy. Sudden as well as gradual morphological changes in the vestibular sensory epithelium are described. Damage to the connective tissue, normal blood vessels and nerve fibres characterized the chronic phase. Both cochlear morphology and hearing remained normal after irradiation of some behaviorally conditioned animals. The temperature rise in the labyrinth during continuous irradiation was studied. The experiments indicated that the mechanical effects may be more significant than the thermal in explaining the structural changes of the inner ear of guinea pigs. The results are compared with previous extensive experience of ultrasound treatment of patients with Meniere's disease.

High frequency ultrasound can cause damage to biological tissues. There is a considerable variation between the susceptibility of different tissues to the effects of ultrasound. Skin, bone, connective tissue and blood vessels are affected far less than nerve fibres and sensory cells. Of all human tissue, nerve tissue is said to be the most sensitive to ultrasound (Fry & Fry, 1953, Åström et al., 1961). This characteristic has been utilized in the treatment of Meniere's disease (Arslan, 1955, James et al., 1960, Sjöberg et al., 1963, Sørensen, 1970, Dionne et al., 1972, Basek, 1973). When aimed at the vestibular part of the inner ear, reduction of the caloric response

and improvement of the vertigo have also been achieved.

Recently, we reported (Stahle, 1976) freedom from vertigo in 52% of the patients following one irradiation. Complete cessation or a considerable decrease of vertigo was reported in 72% by Sørensen (1970). Basek (1973) using the round window technique according to Kossoff et al. (1967) has reported success in 80%. The main objection against the ultrasound method has been the tendency to postoperative hearing deterioration, which in spite of precise aiming of the beam has occurred in 35-40% of the cases (James, 1969, Stahle, 1976).

Several experimental studies on the effects of ultrasound on the inner ear have been performed in various species (James et al., 1964, Stahle, 1964, Dominok & Preibisch, Effenberg, 1965, Giancarlo et al., 1965, Lundquist et al., 1971, Crysdale & Stahle, 1972, Stahle & Sugar, 1973). Degenerative changes have been found in sensory and supporting elements, secretory cells, blood vessels and nerve fibres. Collapse of the utricle and obstructive fibrosis of the semicircular canals have been reported (Lundquist et al., 1971, Barnett et al., 1973), as well as alterations in  $\text{Na}^+$  and  $\text{K}^+$  concentrations in the endolymph (James et al., 1964). The acute morphological changes in the vestibular end

This work was supported by the Swedish Medical Research Council (Project No. B77 17X 3908-05 and B77 7X-03915-05).

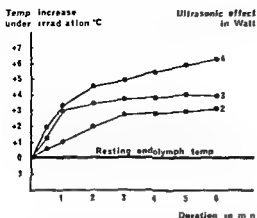


Fig 1 With micro-thermoelements the endolymphatic temperature was recorded continuously during the ultrasonic irradiation. With an energy of 2 W a steady state temperature was reached 2.5°C above the resting temperature and with 3 W the temperature was elevated 3.5°C. With 4 W however there was a continuous increase in the temperature and no steady state occurred within the normal experimental range.

organs have been ascribed to mainly the complex mechanical effects of ultrasound (Lundquist et al, 1971).

The present study presents the morphological characteristics of the guinea pig labyrinth during the period 1–6 days, as well as 6–12 weeks, post irradiation. In order to get an idea of the thermal component of ultrasound, temperature measurements have been made in the inner ear during irradiation. Hearing tests before and after irradiation on behaviorally conditioned animals are included.

## METHODS

The 1.25 MHz 'Ultrapoint' apparatus (Johnson, 1967) was used. The transducer was equipped with a cone shaped steel tip having an end diameter of 1.5 mm which was aimed at the bony wall of the lateral semicircular canal exposed through a postauricular approach. No thinning of the otic capsule before irradiation was been done. The effect was 3 watts and the irradiation time 3 min in all experiments.

For comparison it must be mentioned that in a previous study we (Lundquist et al,

1971) used the Bristol Elliot 3 MHz machine. This has an output effect of around 0.6 W. The area of the tip of its probe is 0.03 cm<sup>2</sup> and the intensity 22 W/cm<sup>2</sup>.

In the present study, where we have used the Ultrapoint machine, the probe's tip area was 0.018 cm<sup>2</sup> and the intensity about 17 W/cm<sup>2</sup>. This means that a much higher effect has been used in this study than in the previous study, mentioned above. Eight guinea pigs were used for histology (light microscopy and EM). One of them was sacrificed immediately after irradiation, the remaining between 2 hours and 6 days after irradiation. Two behaviorally conditioned guinea pigs were irradiated in the same manner; the survival times, however, set to 6 and 12 weeks. Three animals were used for intralabyrinthine temperature recording. In 2 animals the thermocouples (0.25 mm) were inserted in the lateral and anterior ampullae through minute openings in the otic capsule. In one animal the thermocouples were inserted through the oval and round windows. A 12 channel recorder was used to display the inputs from the thermocouples. Several recordings were made simultaneously from each animal.

## RESULTS

### Temperature Measurements

In the animals used for intralabyrinthine temperature recording, several runs were obtained with energy levels ranging from 2–4 W (Fig 1). After 2–3 min of irradiation a plateau was reached at an increased temperature of +3.5°C over the resting endolymph temperature with 2 W, and +3.5°C with 3 W. At an energy of 4 W, however, there was a continuous increase in the temperature, after 6 min becoming more than 6°C above resting temperature.

### Morphology

#### Acute changes

**Light microscopy** 0–2 hours after surgery the appearance of the lateral crista ampullaris was very similar to that of a normal specimen.

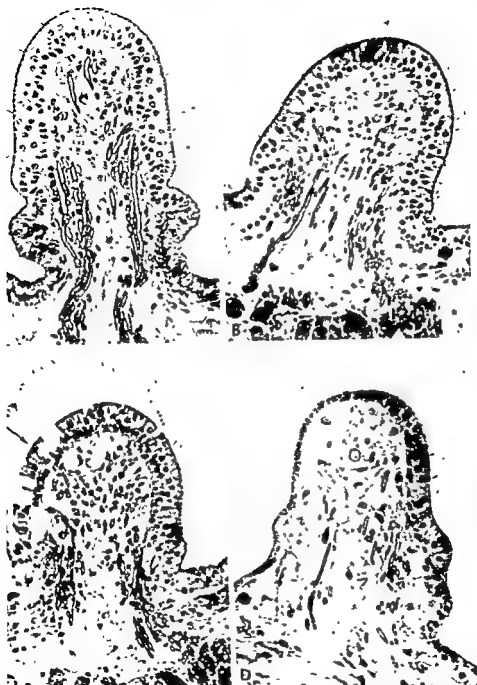


Fig. 2. Ultrasonic irradiation of the lateral semicircular canal ampulla with 3 W for 3 min. (A) Control side with a normal crista. (B) 2 hours after surgery a slight formation of vacuoles and a clearing of the connective tissue closest to the basement membranes are the subtle signs of denervation. (C) Severely disrupted epithelium 2 days after surgery. The canal-side of the crista which has

faced the ultrasonic transducer, shows the most severe damage (arrow). (D) 6 days after surgery the crista is healed, with a cuboidal epithelium covering the region devoid of sensory cells. The connective tissue appears irregular and a reduction of nerve fibres seems to have occurred.

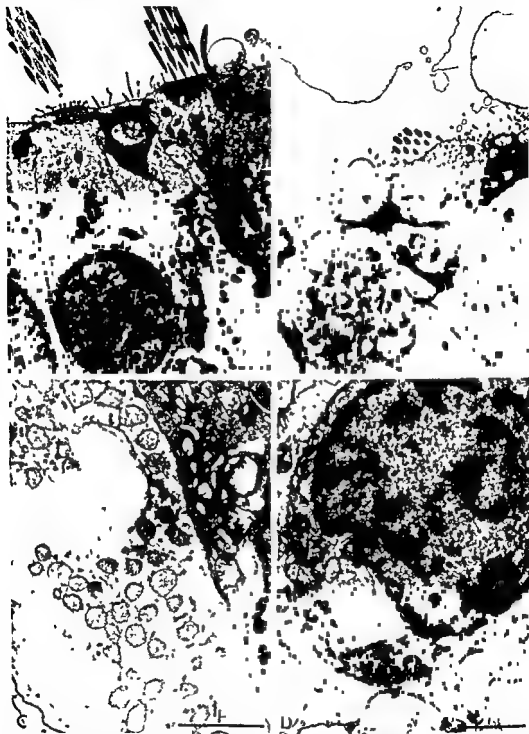


Fig 3 (A) Two hours after surgery Control side with a normal sensory cell type II as well as secretory cells (B) The signs of degeneration, 2 hours after surgery, are intracytoplasmic, with clearing between the mitochondrial cristae as well as formation of vacuoles in the apical cytoplasm (C) Also in the secretory cells (left) changes in

the cytoplasm are present with clear zones and irregularities of the granules. The adjoining sensory (right) demonstrates a much more marked mitochondrial degeneration (D) Irregularities of the cytoplasmic membrane with infoldings of joining cells can be observed below the nucleus in this cell

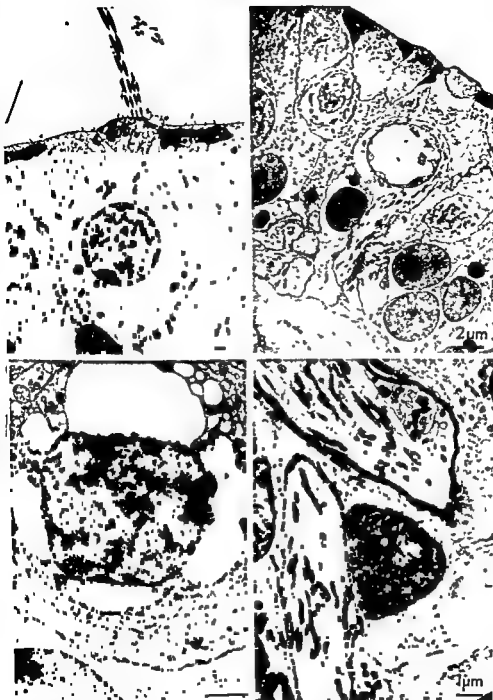


Fig. 4 (A) One day after surgery. Control side with normal sensory cell type I. (B) Severely damaged epithelium with only few sensory cells remaining, one of them appearing completely vacuolarized, but still partly remaining inside the epithelium lining and the surrounding nerve chalice. (C) Some sensory cell nuclei are pyknotic and surrounded by an increasingly vacuolar cytoplasm. (D) The nerve fibres appear normal.

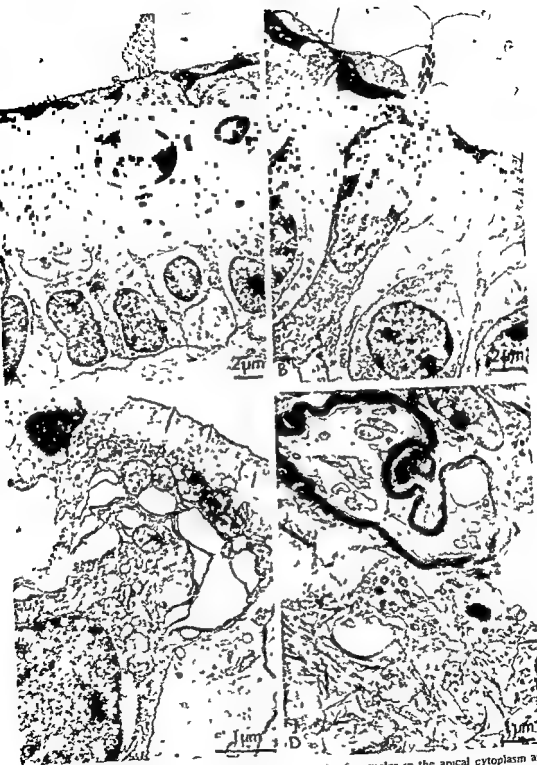


Fig 5 3-6 days after surgery (A) The utricles were normal of vacuoles in the apical cytoplasm and almost normal (B) The only signs of degeneration were the formation of vacuoles in the apical cytoplasm and almost normal (C) Subtle changes could also be found where formation of vacuoles in the apical cytoplasm and almost normal (D) The only signs of degeneration were the formation of vacuoles in the apical cytoplasm and almost normal

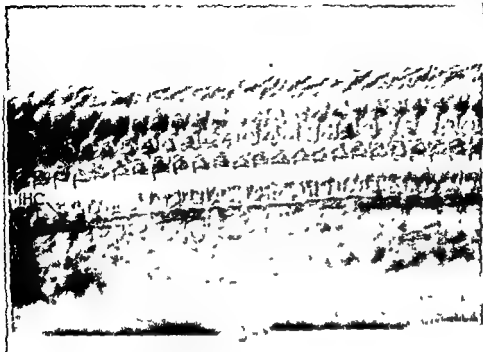


Fig 6 Surface preparation of basal turn cochlea 3 months after surgery, with completely normal sensory cell rows

where the characteristic W shape of the sensory hair bundles can be recognized

There were, however, some signs of vacuolation of the epithelial lining but the sensory cells seemed basically intact. The connective tissue stroma showed a slight edema. The nerve fibres appeared normal (Fig 2B).

**Electron microscopy.** In the sensory epithelium vacuoles were found in the cytoplasm and clear zones appeared in the mitochondria (Fig 3B). The secretory cells also were affected with formation of clear zones in the cytoplasm and granules (Fig 3C). The general cytoarchitecture remained normal, although in some cases a shrinking of the cell was observed and some irregular indentations of the cytoplasmic membrane were present. The nuclei were morphologically normal—that is clumping of the chromatin or other marked changes in the nuclei and nuclear membranes were not observed (Fig 3D).

#### *Subacute changes*

**Light microscopy.** 1–2 days after surgery the picture was markedly changed, characterized

by a disrupted epithelium. Many sensory cells as well as sensory hair bundles had disappeared completely. These findings were more pronounced on the canal side of the crista, closest to the ultrasonic probe (Fig 2C).

**Electron microscopy.** Distinctive from the very acute stage, marked changes now occurred in the cytoplasm and nuclei. Many of the sensory cells were almost completely vacuolized (Fig 4B) or just remained as a cytoplasmic remnant surrounding a pyknotic irregular nucleus. The secretory cells at this stage seemed to be less affected than the sensory cells themselves (Fig 4C). The nerves and nerve-endings were only slightly affected and there were normal appearing mitochondria in the axoplasm (Fig 4D).

#### *Chronic changes*

**Light microscopy.** 3–6 days after surgery the irradiated cristae now seemed to undergo a healing process, with former denuded areas covered with a supporting epithelium devoid



of sensory cells. On the utricular side the sensory cells remained, some of them appearing normal with sensory hair bundles still attached. The connective tissue stroma appeared fibrotic and irregular and there seemed to be fewer nerves remaining.

**Electron microscopy.** On the utricular side of the epithelium where sensory cells still remained, there were signs indicating a continuing process of degeneration. The sensory cells were often markedly shrunken, with a condensed cytoplasm and ruptured mitochondria (Fig. 5B). The nerve chalice surrounding the type I cells seemed swollen with degenerating mitochondria. The most subtle change found at this stage was the formation of vacuoles in the apical cytoplasm and of mitochondria (Fig. 5C).

The connective tissue seemed much more fibrous than in earlier specimens. Few blood vessels remained and the nerves now exhibited an irregular myelin sheath, vacuoles in the axoplasm and irregularities in the mitochondrial cristae (Fig. 5D). The utricular epithelium in the whole series of animals irradiated with 3 W for 3 min was very little affected (Fig. 5A). A detailed analysis remains made, however.

### *Cochlear changes*

In the 3-watt series no cochlear changes were observed, either with behaviour audiometry or with morphology up to 3 months after surgery (Fig. 6).

## DISCUSSION

The biological mode of action of ultrasound is complicated. In general it seems to be based on three concurrent factors—thermal, mechanical, and chemical.

The thermal effect of ultrasound depends on an absorption of energy in tissue. The amount of transmitted sound energy which is converted to heat is determined by the absorption coefficient of the tissue. Bone has a high coefficient and therefore becomes hot (Bender et

al., 1954; Baldes et al., 1958). A 10°C temperature rise in the human otic capsule during ultrasonic irradiation with a strength of 2.8 W has been recorded (Drettner et al., 1967). The substantial heating in the otic capsule in the target area in addition to the structural heating in tissues of varying acoustical impedance inside the labyrinth will raise the intralabyrinthine temperature. In patients undergoing labyrinthectomy, a temperature rise to 40°C was recorded in the vestibule after irradiation with a strength of 2 W during 3 min. In the lateral semicircular canal close to the irradiation spot the temperature rose to 44°C after 3 min irradiation with a strength of 2.5 W (Drettner et al., 1967).

The temperature rise of 2–3°C within 3 min in our experiments on guinea pigs can hardly explain the acute degenerative processes in the crista epithelium. The critical temperature of the facial nerve in man is said to be about 46°C (James et al., 1964). The resistance to heating in neural tissue is further exemplified by the studies of Andersson et al. (1968) which showed that the sciatic nerve action potential in frogs was reduced by 50% for when the temperature reached the 48–51°C range. Against that background it seems more plausible to us in the present study to attribute the degenerative changes after ultrasonic irradiation mainly to mechanical factors. Such an assumption is supported by the results of Fry & Fry (1953) showing that in experiments on hypothermic frogs the ultrasound produced its effects on nerve cells when the temperature in the cord was under 20°C. They consider that the physical factor which should be considered fundamental in the mechanism is the viscous force acting between the submicroscopic structural components of the protoplasm and the surrounding fluid.

The various effects of ultrasound upon the inner ear in Meniere surgery have been discussed in an earlier publication (Sjoberg et al., 1963). At that time we were inclined to attribute the biological effects mainly to the temperature rise. From later studies we obtained

detailed information on the temperature in its parts of the inner ear, which resulted in a change to shorter irradiation periods (Drettner et al., 1967). By reducing the irradiation time, the thermal influence upon the inner ear declined in importance. As a result, the mechanical forces such as "agitation, cavitation and streaming" have become of more immediate interest as factors causing biological damage to the inner ear (Lundquist et al., 1971).

Simple agitation may cause rupture of materials. Sonic streaming, occurring even at very low intensities, is thought to be one of the most important modes of action in biological material (James, 1963), it ruptures cell membranes—as in blood haemolysis—and breaks down bacterial membranes. At lower intensities, important effects on the  $\text{Na}^+$  and  $\text{K}^+$  'pump' in living cells have been observed and are thought to be involved in improving the mechanism after irradiation for Meniere's disease (Hughes & Chow, 1964).

Cavitation, which takes place at high intensities, means the production of microscopic bubbles of gas or vapour in liquids. This phenomenon occurs because, under certain conditions, such a low pressure may occur in the wave troughs that fluid particles are broken up and a hollow space containing a gas phase is formed. From a morphological point of view it seems to be impossible to clearly separate the cellular effects formed by the different mechanical factors. We have therefore grouped them under the heading "mechanical effects" and our morphological findings will be analysed against that background.

At high energy levels with the ultrasonic probe in direct contact with the endolymphatic space, it is possible to disrupt the cristae completely due to cavitation and streaming (Lundquist, 1971). In the present investigation where the otic capsule was preserved, no such signs were observed, but almost immediately after surgery one could with the electron microscope detect changes inside the mitochondria and also the formation of small

vacuoles in the cytoplasm. These changes most likely seem to be due to the sonic streaming, acting at the transition between the cytoplasm and the mitochondrial membranes. When, however, looking at the changes present at the subacute stage (1–2 days after surgery), it is obvious that the damaged mitochondria are gone. The large vacuoles formed in the cytoplasm of the degenerating and disappearing cells, express the severely altered metabolism of these cells. The changes occurring in the nerve fibres are most likely due to the interstitial changes in the connective tissue such as fibrosis and diminished blood supply. Even in the chronic stage (3–6 days after surgery), several severe signs of pathology are present in the sensory cells. Some of the cells are very condensed in appearance and almost all their mitochondria are vacuolar. In other cells the dominant findings are vacuoles formed in the cytoplasm as described in the immediate postoperative state. This varying degree of degeneration occurring in the same sensory cell region is most likely due to an altered cellular metabolism as a result of the mechanical forces of ultrasound. It seems clear from our present results that the ultrasonic irradiation not only gives rise to acute changes in the sensory epithelia but also to progressive changes commencing for several weeks after surgery.

Our findings on experimental animals contribute to the explanation of the favourable effects—as well as the failures—on Meniere patients. Three events can be assumed to take place in the inner ear of man after irradiation: (1) degeneration of the neuro-epithelium and its supporting structures mainly in the non-acoustic part, (2) degeneration of the secretory epithelium at the base of the cristae and (3) the creation of a small fistula in the wall of the membranous labyrinth. All the three, separately, will be able to reduce the symptoms caused by the endolymphatic hydrops. A combined effect, however, seems to be more likely.

(1) The reduction of the caloric reaction

after ultrasonic irradiation recorded in 70% of the Meniere patients (Stahle, 1976) can be explained by general degeneration of the cristae ampullares. The reduction of the caloric response contributes to the diminishing of the vertigo. Our experiments on guinea pigs have verified the existence of partial degeneration. In 2 animals an almost complete degeneration of hair cells on the irradiated side of the crista was found after 6 days, while on the non irradiated side the cell population remained with minor changes visible in light microscopy. Such a selective elimination of sensory cells on the cristae may explain why many patients have been improved though not completely free from vertiginous attacks.

(2) A certain amount of endolymph and mucopolysaccharides is thought to be secreted from the cells in the transitional zone round the base of the cristae. The degeneration of this epithelium following irradiation thus may contribute to the prevention of a labyrinthine hydrops. Degeneration of the transitional cells, with formation of vacuoles and watery appearing cytoplasm, were reported in a previous experimental study on the immediate effects of ultrasound (Lundquist et al., 1971).

Changes in this area have been confirmed in the actual investigation. The importance of such a partial elimination of secretory epithelium in the prevention of a general labyrinthine hydrops remains obscure.

(3) Morphological evidence of the creation of a permanent fistula through the membranous labyrinthine wall at the site of the irradiation has been presented by Sørensen (1976). Similar effect has been attributed to cryo surgery (Wolfson, 1976). We have not analysed our specimens from that particular point of view but concentrated our interest upon the end organ morphology.

The postoperative hearing loss found in around 40% of our patients with follow up time from one to ten years is thought to be the great shortcoming of the ultrasound method. This deterioration is attributable partly to cochlear damage due to ultrasound but

is also an expression of the natural course of the disease (Stahle, 1976). In experimental animals the extensive ampullar lesions after sonic irradiation have not been followed by cochlear lesions. Two behaviorally conditioned animals have presented normal hearing and normal cochlear light microscopy and 12 weeks postoperatively in spite of degeneration in the non acoustic labyrinth. Crude methods of evaluating changes in hearing acuity of research animals have not been taken into account. Possibilities remain however, that a real difference in sensitivity to ultrasound exists between the normal animals in guinea pigs and the diseased end organ in Meniere patients.

## ZUSAMMENFASSUNG

Die Auswirkungen auf die Innenohrstrukturen nach frequenter Ultraschallbestrahlung des lateralen Labyrinths wurden mit Licht und Elektronenmikroskopie analysiert. Sowohl akute als auch progressive morphologische Veränderungen im sensorischen Epithel werden beschrieben. Die Schädigung des Gewebstromas der Blutgefäße und Nervenfasern charakteristisch für die chronische Phase der Erkrankung und das Gehör verblieben unbeändert nach Bestrahlung einiger gehortrauerter Versuchstiere. Der Temperaturanstieg im Labyrinth während der Bestrahlung fortlaufend beobachtet. Die Ergebnisse deuten darauf hin, daß mechanische Effekte die Klärung der Strukturveränderungen im Innenohr von Meerschweinchen bedeutungsvoller sind als bei Mäusen. Die Ergebnisse wurden mit früheren umfangreichen Erfahrungen mit Ultraschallbehandlung von Patienten mit Menierescher Krankheit verglichen.

## REFERENCES

- Andersson T O, Wakin K G, Herick J F, Nett W A & Krusen F H 1951 An experimental study of the effect of ultrasonic energy on the part of the spinal cord and peripheral cord. *Arch Phys Med Rehab* 32: 71.
- Arslan M 1955 Applikation der Ultraschalles auf Labyrinth. Ein Beitrag zur Therapie der Labyrinthose. *Arch Ohren Nasen Kehlkopfheilkd* 167.
- Baldes E J, Herick J F & Stroebe C F 1958 Biologic effects of ultrasound. *Am J Phys Med* 37: 11.
- Barnett S H, Kossoff G & Clark G M 1961 Histological changes in the inner ear of sheep following a round window ultrasonic irradiation. *J Laryngol Soc Austral* 3: 508.

- Casek M 1973 Ultrasound for Meniere's disease *Arch Otolaryngol* 97 133
- Anderson L F, James J M & Herneck J F 1954 Histologic studies following exposure of bone through ultrasound *Arch Phys Med Rehab* 35 555
- Arysdale W M & Stahle J 1972 Ultrasonic irradiation of the guinea pig cochlea *Ann Otol Rhinol Laryngol* (St Louis) 81 87
- Barber J, Barber H & Briant T D R 1972 Results of ultrasound therapy for Meniere's disease *Can J Otolaryngol* 1 6
- Cominok G W & Preibisch Effenberger R 1965 Die histologischen Veränderungen am Labyrinth des Kaninchens nach experimentellen einseitiger Vestibularisausschaltung mit Ultraschall *Z Laryngol Rhinol Otol* 44 9
- Freundlich H F, Johnson S, Sjöberg A & Stahle J 1967 Some thermal effects of ultrasound on the inner ear *Acta Otolaryngol* (Stockh) 64 464
- Fry W J & Fry R B 1953 Temperature changes produced in tissue during ultrasonic irradiation *J Acoust Soc Am* 25 6
- Fry W J, Barnard J W, Fry F J & Breman J F 1955 Ultrasonically produced localized selective lesions in the central nervous system *Am J Phys Med* 34 413
- Gancarlo H, Choo Y B, Wolff D, Bisi R H & Weymuller E A 1965 Vestibular changes following ultrasonic irradiation *Arch Otolaryngol* 82 365
- Hughes D E & Chow J T Y 1964 The biochemistry of the inner ear and the consequences of treatment by ultrasound *Acta Otolaryngol* (Stockh) Suppl 192 199
- James J A 1963 New developments in the ultrasonic therapy of Meniere's disease *Ann Roy Coll Surg Engl* 33 276
- James J A 1969 Meniere's disease: treatment with ultrasound *J Laryngol Otol* 83 771
- James J A, Dalton M A, Bullen M A, Freundlich H F & Hopkins J C 1960 The ultrasonic treatment of Meniere's disease *J Laryngol Otol* 74 730
- James J A, Dalton G A, Freundlich H F, Bullen M A, Wells P N T, Hughes J A & Chow J T Y 1964 Histological thermal and biochemical effects of ultrasound on the labyrinth and temporal bone *Acta Otolaryngol* (Stockh) 57 306
- Johnson S 1967 An ultrasonic unit for the treatment of Meniere's disease *Ultrasonics* 5 173
- Kossoff G, Wadsworth J R & Dudley P F 1967 The round window ultrasonic technique for treatment of Meniere's disease *Arch Otolaryngol* 86 535
- Lundquist P M, Igarashi M, Wersall J, Guilford F R & Wright W K 1971 The acute effect of ultrasonic irradiation upon ampullar sensory epithelia of the guinea pig *Acta Otolaryngol* (Stockh) 72 111
- Sjöberg A, Stahle J, Johnson S & Sahl R 1963 Treatment of Meniere's disease by ultrasonic irradiation *Acta Otolaryngol* (Stockh) Suppl 178
- Sørensen H 1970 The clinical effect of labyrinthectomy and ultrasonic irradiation in Meniere's disease. In *Vestibular Function on Earth and in Space*. Wenner Gren symposium series No 15 (ed J Stahle) p 135. Pergamon Press, Oxford & New York, 1970.
- 1976 The effect of ultrasound on Meniere's disease *Acta Otolaryngol* (Stockh) 87 312
- Stahle J 1964 Some effects of ultrasound on the inner ear *Acta Otolaryngol* (Stockh) Suppl 197 197
- 1976 Ultrasound treatment of Meniere's disease. Long term follow up of 356 advanced cases *Acta Otolaryngol* (Stockh) 81 170
- Stahle J & Sugar J 1973 The stria vascularis after ultrasonic irradiation *Equilibr Res* 3 130
- Wolfson R J 1976 Labyrinthine cryosurgery for Meniere's disease. Proc Barány Soc Vth extraordinary meeting Kyoto Oct 17-21 1975 (ed M Morimoto) p 298
- Åström K E, Bell E, Ballantyne Jr H T & Heidensleben E 1961 An experimental neuropathological study of the effects of high frequency focussed ultrasound on the brain of the cat *J Neuropath Exp Neurol* 20 484

P G Lundquist MD  
Dept of Otolaryngology  
Karolinska sjukhuset  
S 104 01 Stockholm  
Sweden

## THE EFFECTS OF MERCURIAL POISONING ON THE VESTIBULAR SYSTEM

M. Anniko and L. Sarkady<sup>1</sup>

From the Department of Otolaryngology, Karolinska sjukhuset, Stockholm, and King Gustaf V Research Institute, Karolinska institutet, Stockholm, Sweden

(Received January 19 1977)

**Abstract** The sensory epithelium with adjacent nerve endings and the secretory epithelium may both become damaged following mercury chloride intoxication. Peripheral myelinated nerve fibres in the crista ampullaris showed signs of degeneration following chronic poisoning. Ultrastructural alterations of the vestibular hair cells initially occurred in animals free from clinical signs of intoxication. The sensory epithelium and occasionally the secretory cells were affected before signs of ultrastructural damage could be detected in the myelinated nerves

et al., 1975, v. Strupler, 1952). The increase in environmental pollution has further focused interest on the possible toxic effects of mercury.

The purpose of the present study was to investigate whether mercury can cause vestibular dysfunction by damage to the sensory or the secretory epithelia of the cristae ampullares.

Neuro-otological disturbances following the use of drugs containing mercury have been known since antiquity. Kussmaul (1861) reported vestibular dysfunction as one of the main symptoms following mercury poisoning. Disease caused by mercury compounds used to be counted as a clinical rarity until several recent outbreaks of mass poisoning aroused public concern over its dangers (Goldwater, 1972, Takeuchi, 1972).

Although many reports concerning mercury poisoning have appeared recently, there have been only a few reports of neuro-otological observations on the intoxication caused by mercurials (Fryisaki et al., 1971; Mizukoshi

### MATERIALS AND METHODS

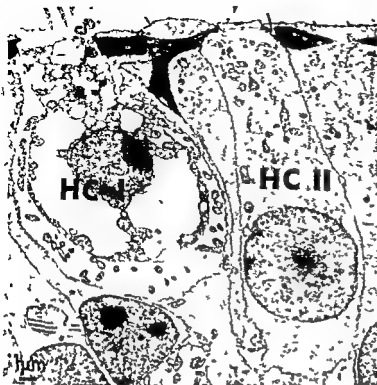
Sixty healthy young guinea pigs (250-350 g) were used in this study. The animals were treated with daily subcutaneous injections of mercury chloride ( $\text{HgCl}_2$  as a 0.5% solution in sterile water) in doses ranging from 2.5 to 25 mg/kg b.w. (chronic intoxication: 2.5-7.5 mg/kg b.w., acute intoxication: 7.5-25 mg/kg b.w.). The total dose varied between 10 and 90 mg/kg b.w.

The survival time after the last injection varied from 6 hours to 1 month. The control group of animals consisted of 10 healthy untreated guinea pigs.

The clinical investigation of the animals and the procedure for fixation and morphological investigation have been described earlier by Anniko & Sarkady (1977) concerning cochlear pathology following exposure to mercury chloride.

Supported by grants from Karolinska Institutet and the Swedish Medical Research Council (No. 12X-00720).

<sup>1</sup> Then visiting scientist at the Department of Otolaryngology, Karolinska sjukhuset. Present address: Department of Otolaryngology, Semmelweis University Hospital, Budapest, Hungary.



*Fig 1* EM Chronic poisoning  
Vesicular degeneration of hair cell type I (HC I) while the hair cell type II (HC II) still is rather unaffected by the toxic effect(s) of mercury chloride

## RESULTS

### *Sensory epithelium*

In the investigated specimens of both types of poisoning, damage to hair cells type I and type II occurred. However, acute intoxication affected the hair cells to a much lesser extent than chronic intoxication, in which case hair cells were frequently found in various stages of cellular disintegration. The individual susceptibility of the hair cells to mercury chloride poisoning was, however, initially great because undamaged hair cells often appeared adjacent to severely degenerating hair cells of both types (Figs 1 and 2).

A complete loss of hair cells in the crista ampullaris or the macula utriculi did not occur. Hair cells of type I were morphologically often affected before those of type II (Fig 1). In hair cells of type I the nuclear membrane sometimes appeared as contiguous with a membrane system entering the cellular cytoplasm and electron-dense material was found

within this membrane system giving the appearance of a deposit which had been ejected from the nucleus. Finally, both types of damaged hair cells showed vesicular degeneration of the cytoplasm and the cellular organelles. The cytoplasm in some sensory cells, mostly type I hair cells, showed increased electron density. A large number of vesicles of varying size appeared (Fig 3 A, B), as a result of which the cytoplasm became fragmented. The nuclear membrane appeared wavy but was not discontinuous.

The mitochondria were affected at an early stage of cellular damage and in many specimens the mitochondrial changes appeared as the first signs of hair cell pathology. The mitochondria in the hair cells and the nerve endings showed similar structural changes. In some mitochondria the two lamellae of a crista normally in close apposition appeared initially to be separate from each other, thus forming a minimal vacuole in the matrix space. The cristae later fragmented without the formation

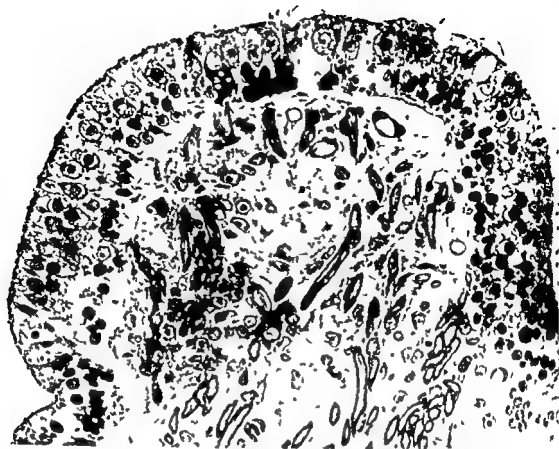


Fig 2 Light microscopy (LM) Crista ampullaris. Degenerating hair cells of both types. A dark osmophilic

inclusion (arrow) is found below a hair cell (degenerate nerve ending?)  $\times 120$

Myelin figures and large, confluent, non-stainable regions of the mitochondrial matrix occurred, giving an empty appearance. The mitochondrial outer membrane often showed herniations or even ruptures, a phenomenon not observed in the control material which underwent the same morphological preparation procedure.

Following chronic intoxication, afferent and efferent nerve endings revealed signs of damage when the adjacent hair cell sustained ultrastructural damage (Fig 3 C, D). It proved impossible, however, to establish which was damaged first, the sensory cell or the nerve endings. Some of the nerve chalyces of type I hair cells became opaque, as when a deposit of mercury compound is found in the neuroplasm. In other, less damaged afferent nerve terminals, the mitochondria appeared swollen

with destruction of cristae. Similar morphological changes also occurred in efferent nerve endings, while the amount of transmission vesicles was estimated to be normal for a long time. Nerve terminals adjacent to type II sensory cells were affected at the same time. Myelinated nerve fibres innervating type I hair cells and the cytological changes were similar. Myelinated nerve fibres in the connective tissue of the crista ampullares were often damaged following chronic poisoning (Fig 4) but the degree of the structural vulnerability varied greatly. Ultrastructurally normal myelinated nerves could be found in the same specimens as myelinated nerve fibres in different stages of dissolution. In the degenerating nerves the organelles of the axoplasm were reduced in number or even lost completely, while the myelin sheaths disintegrated (Fig 4 A, B). Axons often contained

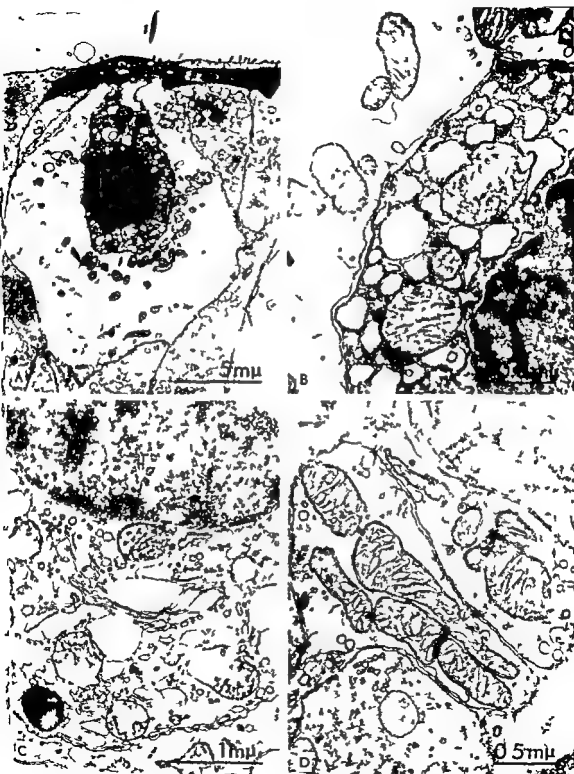


Fig 3 Electron microscopy (EM) Crista ampullaris A Chronic intoxication. Type I hair cell showing an electron dense cytoplasm containing vacuoles of varying size. The adjacent afferent nerve chalice is widened but still contains many mitochondria B Detail from Fig 1A C

Acute intoxication. The cytoplasm of a type I hair cell is vesiculated and the mitochondria have lost their cristae formation D Acute intoxication. Afferent nerve ending. The mitochondrial cristae appear separated from each other





Fig. 4. EM. Chronic intoxication. Myelinated nerve fibres. The axons contain foci with floccular dense bodies and granular debris.

with floccular dense bodies and granular debris.

In acute intoxication both types of sensory cells were affected only very slightly while adjacent nerve terminals could appear ultrastructurally changed. However, the individual susceptibility of the nerve endings to mercury chloride poisoning varied; thus both normal and damaged nerve endings were observed in the same specimen. Some specimens even appeared completely normal. The variation in the nerve ending damage to individual nerve terminals within one specimen was relatively large, thus enabling us to study the course of the destruction of the nerve ending under observation.

When afferent and efferent nerve endings were damaged, no difference in sensitivity could be observed between the two types. The mitochondria were changed in the same

way as described concerning the hair cells. Acute intoxication did not affect myelinated nerve fibres.

#### Secretory epithelium

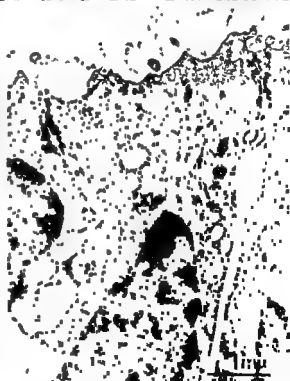
Chronic mercury intoxication can sometimes cause alterations in the ultrastructure in dark cells of the secretory epithelium. Administration of small amounts of mercury each injection (2.5 mg/kg b.w.) did not result in any changes other than an increased proliferation of the dark cells, normally containing secretory granules.

Treatment with 5.0 mg/kg b.w. on occasion caused after 5–16 injections widening of the space between the long digitations of the dark cell membrane which contain elongated mitochondria. This widening could become so extensive that the cells appeared as almost separate from



**Fig 5** EM Chronic intoxication Dark cells of the secretory region of the crista ampullaris. The cellular extensions are separated and the dark cells normally adjacent to each other show large intercellular regions except at the surface area towards the endolymphatic space

**Fig 6** EM Chronic intoxication Oedema in cells of the



secretory epithelium with a lighter cytoplasm than surrounding dark cells

**Fig 7** EM Chronic intoxication Detail from the apical part of a dark cell showing an increase in electron density

other except at the margin towards the endolymphatic space (Fig 5) and the dark cell cytoplasm increased in electron density (Fig 7). Severely degenerating dark cells were not observed and ejection of damaged secretory cells into the endolymphatic space did not occur. However, vacuoles of considerable size frequently occurred.

Sometimes cystic formations were found below the secretory area of the dark cells. Infrequently, cells with a lighter cytoplasm than surrounding dark cells were interspersed in the secretory region, in which case their cytoplasm appeared severely oedematous and lipid like inclusion bodies could be observed (Fig 6).

Acute intoxication affected the dark cells of the secretory region only in rare cases, but when this occurred the morphological changes were similar to those found following chronic intoxication.

The supporting cells or the secretory cells between the hair cells in the sensory epithelium were structurally unchanged. There were likewise no changes in the transitional cells between the sensory area and the dark cells.

## DISCUSSION

Clinical observation of neuro otological disturbances following chronic poisoning by organic mercurials has revealed frequent signs of dys-equilibrium as one of the main findings. Mizukoshi et al (1975) reported from their studies on 144 patients with Minamata disease following the Aganogawa River mass poisoning (organic mercurials) that signs and symptoms of disturbance of the vestibular system occurred in 90% of the investigated cases, thus being a frequent symptom in mercury poisoning. Similar observations were made by Kurland et al (1960) concerning the Minamata Bay poisoning in the 1950s.

The present ultrastructural study of the vestibular system showed that damage to both types of hair cells may occur, but there was a great variation in individual susceptibility to

the toxic effect(s) of mercury chloride. The secretory epithelium was seldom affected and the supporting tissue remained morphologically normal. When damage occurred, the terminals degenerated at the same time as the adjacent hair cells.

The serious symptoms of toxicity arise from chronic occupational exposure to organic mercury and exposure to alkyl mercury compounds result from damage to the nervous system (McGregor & Clarkson 1974). It is generally assumed that the pathological effects produced by mercurials result from alterations in SH containing macromolecules within or on the surface of the target (Hughes, 1957; Passow et al, 1961; Rothstein, 1973).

The distribution of mercury within cells has been reviewed by Hopwood (1973) who reported that mercury binds very rapidly to the cell membranes, so that the reaction is completed within 2-3 minutes, suggesting the formation of typical covalent bonds. A leakage occurred of potassium ions from within the cells and sodium ion transport was inhibited. The cellular uptake of glucose and glycine ceased. Once the mercuric chloride is within the cell it will be distributed between the various components of the cytoplasm and the nucleus. Norseth (1968) reported that the nuclei and lysosomes take up a considerable portion of added mercury. Venty & Reijnders (1967) found that both organic and inorganic mercuric compounds destroyed the lysosomal membrane, with a subsequent release of bound lysosomal enzymes into the cytoplasm. However, Venty & Brown (1970) reported that mercuric chloride inhibited the activity of acid hydrolases released from the lysosomes. Findings of metallic mercury with the lysosomes of macrophages have been reported by Burge & Winkelmann (1970).

In the present investigation, degeneration of the sensory epithelium has been found in most specimens in which the dark cells of the secretory region engaged in the active and passive transport of ions for the preservation of

normal composition of the endolymph appeared normal. This indicates a direct action of mercury chloride on the sensory cells. Mitochondria were affected early both in the nerve endings and in the adjacent hair cells. Hopwood (1973) reported that the overall effect of mercurials was to inhibit the respiration of the cell thus primarily affecting the mitochondria. Reos et al (1967) showed that mercurial compounds give rise to mitochondrial swelling. Brierly et al (1967) found that the swelling of the mitochondria caused by mercurials was due to a passive permeability to chloride ions. They also found that mitochondria bound mercurials in proportion to the hydroxyl ion concentration (Jacobus & Brierly 1969).

Damage to nerve endings and myelinated nerve fibres was a prominent feature of the present investigation. The difference between chronic and acute intoxication can probably be explained by the difference in the concentration and the duration of the exposure. Relatively large amounts of mercury introduced suddenly may affect the excitable membrane directly whereas the sustained presence of smaller concentrations may lead to an accumulation of the poison within the cells and to a consequent disorder of metabolism.

Annko & Sarkady (1977) interpreted some of their findings in myelinated cochlear nerves following chronic mercury chloride intoxication as a sign of demyelination. According to Herman et al (1973) demyelination is not a morphological feature in mercury poisoning. However Chang & Hartmann (1972) interpreted some of their findings as indicative of a demyelinating process. Axonal degeneration with a loss of organelles in myelinated nerves has been reported in a number of conditions many apparently related to a suppression of oxidative energy metabolism including cyanide intoxication (Hirano et al 1967; Hirner 1969) and hypoglycemia (Webster & Ames 1965). Suppression of oxidative phosphorylation has been reported following mercury and other heavy metal administration causing swelling of the mitochondria (Southard et al

1974). While an axon is degenerating it becomes filled with floccular dense bodies and granular debris (Hirano 1972). Dense bodies consisting of lipid accumulations have been reported also in a number of lipoidoses (Gonatas et al 1968; Guazzi & Van Bogaert 1969).

Pathological findings in the vestibular examination of patients with clinical signs of mercury intoxication have revealed a high frequency of gaze nystagmus (17%) and direction changing positional nystagmus (65%) (Mizukoshi et al 1975). It was suggested that these nystagmus findings may be associated with diffuse lesions of the brain stem and the cerebellum. Irregular nystagmus was observed also by Hunter & Russel (1954) though they did not discuss the underlying pathology.

The present experimental study reveals that the vestibular hair cells of both types with adjacent nerve endings may become damaged following both acute and chronic intoxication. Damage to the sensory epithelium occurred in the early stages without clinical signs of vestibular dysfunction. The hair cells with adjacent nerve endings when damaged appeared so before the myelinated nerves became affected indicating a primary effect in the crista ampullaris.

## ACKNOWLEDGEMENT

The authors wish to express their gratitude to Professor Jan Wersäll, Department of Otolaryngology, Karolinska sjukhuset, for valuable advice and for critical revision of the manuscript. The technical assistance of Mrs Marie Louise Spångberg, Miss Wiveca Ring, Miss Lena Hermanson and Mr Bengt Hedberg is gratefully acknowledged. The manuscript was linguistically reviewed by Mrs Ann Kempe.

## ZUSAMMENFASSUNG

Das sensorische Epithel um mit den angrenzenden Nervenenden sowie das sekretorische Epithel um können bei der Zufolge von Mercurum-Chloridvergiftung beschädigt werden. Peripherische bemerkte Nervenfasern in der Crista ampullaris waren auch Zeichen von Degeneration auf Ultrastrukturelle Veränderungen in der Crista ampullaris konnten in Tieren die kein Zeichen von Berausung aufwiesen vorkommen. Das sensorische Epithel um wurde beschädigt bevor Zeichen von ultrastrukturellen Veränderungen in den bemerkten Nerven entdeckt werden konnten.

## REFERENCES

- Anniko M & Sarkady, L 1977 Cochlear pathology following exposure to mercury chloride *Acta Otolaryngol* (Stockh) In press
- Arcos, J C, Stacey, A, Mathison, J B & Argus, M F 1967 Kinetic parameters of mitochondrial swelling *Exp Cell Res* 48, 448
- Brierly, G P, Settlemyre, C T & Knight, N A 1967 Induction of  $K^+$  transport and swelling in isolated heart mitochondria by mercurial compounds *Biochem Biophys Res Comm* 28 420
- Burge, K M & Winkelmann, R K 1970 Mercury pigmentation, an electron microscopic study *Arch Derm* 102, 51
- Chang L W & Hartmann, H A 1972 Ultrastructural studies of the nervous system after mercury intoxication II Pathological changes in the nerve fibres *Acta Neuropathol* 20 316
- Fryisaki, R, Ohno, Y & Ohtake, K 1971 Hearing disturbance in chronic intoxication with organic mercury *Audiology Japan* 14, 484
- Goldwater, L 1972 *Mercury*. York Press, Baltimore, USA
- Guazzi, G C & Van Bogaert, L 1969 *In The Structure and Function of Nervous Tissue* (ed G H Bourne), vol 3, pp 383 Academic Press, New York/London
- Gonatas, N K, Gambetti P & Baird, H 1968 A second type of late infantile amaurotic idiocy with multilamellar cytosomes *J Neuropathol Exp Neurol* 27, 371
- Herman S P, Klein, R, Talley, F A & Kngman, M R 1973 An ultrastructural study of methyl mercury induced primary sensory neuropathy in the rat *Lab Invest* 28 104
- Iirano, A 1972 The pathology of the central myelinated axon *In The Structure and Function of the Nervous System* (ed G H Bourne), vol 5 structure III and physiology III Academic Press Inc New York/London
- Hirano, A, Levine, S & Zimmerman, H M 1967 Experimental cyanide encephalopathy Electron microscopic observations of early lesions in white matter *J Neuropathol Exp Neurol* 26 200
- Hirner, A 1969 Elektronenmikroskopische Untersuchungen zur formalen Genese der Balkenläsionen nach experimenteller Cyanvergiftung *Acta Neuropathol* 350
- Hopwood D 1973 Fixation with mercury salts *Acta Histochemica Suppl Band XIII* 107
- Hughes W L 1957 A physicochemical rationale for the biological activity of mercury and its compounds *Ann N Y Acad Sci* 65 454
- Hunter, D & Russel B 1954 Focal cerebral and cerebellar atrophy in a human subject due to organic mercury compounds *J Neurol Neurosurg Psychiatr* 17, 235
- Jacobus W E & Brierly, G P 1969 Ion transport heart mitochondria Cation binding by mitochondrial particles *J Biol Chem* 244 4995
- Kurland, L, Faro, S N & Siedler H 1960 Merc disease *World Neurology* 1 370
- Kussmaul, A 1861 Untersuchungen über den kationellen Mercunismus und seine Verhältnisse konstitutionellen Syphilis Würzburg
- MacGregor, J T & Clarkson T W 1974 Distribution, tissue binding and toxicity of mercurials *Adv Med Biol* 48 463
- Mizukoshi, K, Nagaba, M, Ohno Y, Ishikawa, Aoyagi M, Watanabe, Y, Kato I & Ino H I Neurological studies upon intoxication by organic mercury compounds *ORL* 37, 74
- Norseth, T 1968 The intracellular distribution of mercury in rat liver after a single injection of mercuric chloride *Biochem Pharmacol* 17, 581
- Rothstein A 1973 Mercaptans, the biological target mercurials *In Mercury Mercurials and Mercury* (ed M W Miller & T W Clarkson) pp 68-92. Thomas, Springfield, Ill
- Passow, H, Rothstein A & Clarkson T W 1961 general pharmacology of the heavy metals *Pharm Rev* 13 185
- chronischer Quecksilberintoxikation *Pract Otolaryngol* (Basel) 14, 264
- Takeuchi, T 1972 Biological reactions and pathological changes of human beings and animals under condition of organic mercury contamination *Environmental Mercury Contamination* (ed R tung & D B Dinman), pp 247-289 Ann Science Publishers, Michigan, USA
- Venty, M A & Reith, A 1967 Effect of mercurial compounds on structure linked latency of somal hydrolases *Biochem J* 105 685
- Venty, M A & Brown W J 1970  $Hg^{2+}$  induced tubular nephroses *Am J Pathol* 61, 57
- Webster, H DeF & Ames, A III 1965 The ultrastructure and physiology of anoxia and hypoglycemia nervous tissue Proceed V Internat Congr Path Zurich Exc Med Foundat, pp 62-63 Amsterdam (1966)

M Anniko, M D  
Dept of Otolaryngology  
Karolinska sjukhuset  
S 10401 Stockholm 60  
Sweden

## BILATERAL NASAL VASCULAR RESPONSES TO UNILATERAL SYMPATHETIC STIMULATION

H Wilson and M S Yates

*From the Department of Pharmacology and Therapeutics  
University of Liverpool, Liverpool, England*

(Received March 4 1977)

**Abstract** Unilateral preganglionic cervical sympathetic stimulation in the anaesthetized cat evoked vasoconstriction in both nasal cavities dependent on stimulation frequency. Vasoconstriction in the contralateral cavity was 15-20% of that on the stimulated side. Similar findings were obtained on unilateral Vidian nerve stimulation. Vasoconstriction evoked in the sympathetomized nasal cavity by stimulating the opposite cervical chain was reduced but not abolished by sectioning the posterior nasal and ethmoidal nerves of the stimulated side. It is suggested that vasoconstrictor fibres reach the opposite cavity either in these nerves or by way of blood vessels. It is more likely that sympathetic fibres from these pathways innervate blood vessels which supply both nasal cavities.

Bilateral hyperaemia and decreased nasal patency have been reported in man following unilateral cervical sympathectomy and stellate ganglion block (Bertein 1929, Stocksted & Thomsen 1953). In contrast to Undritz (1929) and Anggård & Densert (1974), Franke (1966) and Malm (1973) reported bilateral vasoconstriction and increased patency in the nose of the dog and cat on unilateral cervical sympathetic stimulation. The contralateral responses were between 6 and 12% of the responses in the ipsilateral cavity. Richerson & Seeborn (1968) and Franke (1966) suggested they were probably due to a crossover of sympathetic fibres in the nose.

In the current investigations, the vasoconstriction evoked in both nasal cavities of

anaesthetized cats by unilateral cervical sympathetic stimulation has been examined at different frequencies. Experiments have also been undertaken to determine whether contralateral responses can be evoked on stimulation of the opposite Vidian nerve, which conveys post ganglionic sympathetic vasoconstrictor and preganglionic parasympathetic vasodilator and secretory fibres to the nose (Slome, 1955, Malcomson, 1959, Eccles & Wilson, 1973). Some sympathetic fibres reach the nasal cavity in the ethmoidal and posterior nasal nerves (Wilson & Yates, 1977) and to investigate whether they are involved in the contralateral response, vasoconstriction produced in the sympathetomized nasal cavity by stimulating the opposite cervical sympathetic chain was examined before and after sectioning these nerves.

Some of these findings have already been demonstrated to the Physiological Society (Wilson & Yates 1975).

### METHOD

Cats of either sex 2-4 kg body weight were anaesthetized with pentobarbitone sodium 40 mg/kg I/P and the trachea was cannulated.

Vasoconstriction and vasodilation in the nose were recorded as a decrease or increase in pressure respectively in the sealed nasal cavity by means of a short plastic cannula

This work was supported by a Medical Research Council project grant awarded to Dr H. Wilson.

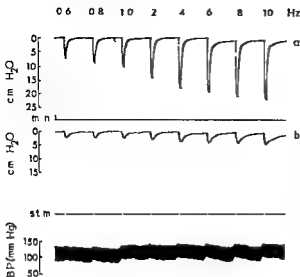


Fig. 1 Vasoconstriction in (a) ipsilateral (b) contralateral nasal cavity on supramaximal unilateral preganglionic cervical stimulation at different frequencies. Lower record shows arterial blood pressure.

which was inserted into the nostril and connected to a pressure transducer (Bell & Howell type 4 327-L223) and a Devices M2 pen recorder (Wilson & Yates, 1975). This method only records the overall vascular changes in the nose and according to Anggård & Edwall (1974) and Malm (1974) it will mainly reflect the changes in the capacitance vessels. When recordings were made simultaneously from both nasal cavities it was ensured that a pressure change in the recording system of one side did not produce a similar change in that of the opposite side.

In one series of experiments the cut peripheral end of the preganglionic cervical sympathetic chain of one side was mounted on bipolar platinum electrodes and covered with small cotton wool plugs soaked in liquid paraffin (B. P.). The opposite preganglionic cervical sympathetic chain was also sectioned and the ends crushed.

In another series of experiments the Vidian nerve was exposed in the orbit of one side (Eccles & Wilson, 1973). The cut peripheral end was placed on bipolar platinum electrodes and covered with a small cotton wool swab soaked in liquid paraffin (B. P.). The posterior

nasal and ethmoidal nerves were also cut in the orbit. The maxillary nerve, the palatine nerves and both cervical sympathetic chains were sectioned.

Square wave impulses were delivered by a Grass S4 stimulator through an isolation unit (SIU4). The preganglionic cervical sympathetic chain was stimulated supramaximally using a pulse width of 0.5 msec. The nerve was stimulated using a pulse width of 1.0 msec at an intensity of 70 V (Eccles & Wilson, 1974). In all experiments the stimulus frequency ranged from 0.1–30 Hz and the nerve was stimulated for 15 sec every 3 min.

In some experiments the superior ganglion of one side was removed under the same technique 10–14 days previously.

In all experiments the arterial blood pressure was recorded from a femoral artery by means of a pressure transducer (Bell & Howell type 4 327-221) and a pen recorder (Devices M2).

## RESULTS

### *Vascular responses evoked in both nasal cavities by preganglionic stimulation of the cervical sympathetic chain*

The preganglionic sympathetic chain was stimulated on one side in 10 cats at frequencies ranging from 0.1–30 Hz and vasoconstriction was recorded simultaneously from both nasal cavities.

Vasoconstriction appeared quickly and simultaneously on both sides and increased with increasing stimulation frequency, the maximum value being attained a few seconds after stimulation began. The minimal effect frequency on both sides was 0.1 Hz. Maximal vasoconstriction occurred between 10 and 30 Hz. Fig. 1 shows the responses obtained in a typical experiment. The contralateral responses were, however, much smaller (Fig. 1b) and at each frequency were between 10 and 20% of the ipsilateral responses (Fig. 1). Vasoconstriction was often accompanied by a rise in arterial blood pressure at the higher frequencies (Fig. 1).

Vasoconstriction in the contralateral nasal cavity still occurred in 2 cats after acute bilateral adrenalectomy

Since the Vidian nerve generally conveys a large proportion of the post-ganglionic sympathetic fibres to the nasal cavity, this nerve was also stimulated to determine whether a vasoconstrictor response could be evoked in the contralateral cavity

#### *Nasal vascular responses evoked by stimulating the cut peripheral end of the Vidian nerve*

The cut peripheral end of the Vidian nerve was stimulated in 4 cats. The vasoconstriction evoked at different frequencies was recorded from the ipsilateral nasal cavity with the contralateral cavity open anteriorly. Responses to these frequencies were then recorded from the contralateral cavity with the ipsilateral cavity open anteriorly.

A vasoconstrictor frequency/response curve of one of these experiments which is typical of the others is shown in Fig 2. At each frequency the ipsilateral responses have been expressed as a percentage of the maximum value observed in each cat and the contralateral responses as a percentage of the maximum ipsilateral response in the same cat. The minimal effective frequency in the ipsilateral cavity was 0.1 Hz, vasoconstriction increased with increasing stimulation frequency, reached 80% of maximum at 3 Hz and the maximum value at 15 Hz. In the contralateral nasal cavity, the minimum effective frequency was 0.2 Hz, the vasoconstrictor responses reaching 80% maximum at 2-4 Hz and the maximum value at 10 Hz, they were only approximately 7-15% of those observed in the ipsilateral cavity.

Since the posterior nasal nerve conveys sympathetic nerves to the nasal cavity, attempts were made to stimulate it as it leaves the sphenopalatine ganglion to enter the foramen but these proved impossible because in all the animals used, the nerve was too short for the application of

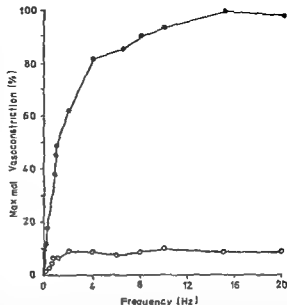


Fig 2 Vasoconstrictor frequency/response curves from ipsilateral (●—●) and contralateral (○—○) nasal cavity on supramaximal unilateral Vidian nerve stimulation in one cat. One nasal cavity open anteriorly during recording from the other. Ipsilateral response expressed as % of maximal vasoconstriction, contralateral responses as % of maximal ipsilateral response.

electrodes. In no instance did Vidian nerve stimulation cause any change in arterial blood pressure.

#### *Vascular responses in sympathectomized nasal cavity*

Degeneration of the sympathetic fibres was confirmed by the finding that stimulation of the cut peripheral end of the Vidian nerve on the sympathectomized side in 7 cats produced vasodilation but not vasoconstriction as in normal animals.

Stimulation of the opposite preganglionic cervical sympathetic chain caused vasoconstriction in the sympathetically denervated nasal cavity. In these experiments the innervated nasal cavity was open anteriorly and posteriorly.

The mean vasoconstrictor frequency/response curve obtained in 7 cats, together with standard error bars is shown in Fig 3. These recordings were made at a higher



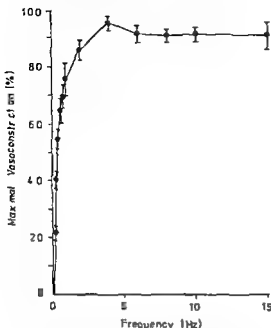


Fig. 3 Vasoconstrictor frequency/response curve with S.E. bars from sympathectomized nasal cavity (10–14 days after removing superior cervical ganglion) on supra-maximal stimulation of pre-ganglionic cervical sympathetic chain of opposite side. Innervated cavity open anteriorly and posteriorly. Each value is the mean of responses in 7 cats. Responses expressed as % of maximal vasoconstriction in each cat.

degree of amplification than the contralateral responses shown in Fig. 2. The minimum effective frequency was 0.1 Hz; the responses were approximately 80% of maximum at 2 Hz and maximum at 4 Hz.

Vasoconstriction still occurred when the posterior nasal nerve on the denervated side was sectioned between the sphenopalatine ganglion and the sphenopalatine foramen. It was reduced, however, by approximately 60% after sectioning of this nerve on the side of stimulation in one experiment and by cutting the ethmoidal nerve on this side in another cat.

## DISCUSSION

The present investigations confirm that when the preganglionic cervical sympathetic chain of one side is stimulated, vasoconstriction occurs simultaneously in both nasal cavities and that it increases with increasing stimulation

frequency. Vasoconstriction in the contralateral cavity is however only approximately 15–20% of that in the ipsilateral cavity. It is unlikely for two reasons that the pressure reduction in the contralateral cavity is due to that caused by vasoconstriction in the cavity on the stimulated side. Firstly, because it was ensured that in the absence of nerve stimulation, a pressure change in one cavity did not affect that in the other, and secondly, because it occurred on Vidian nerve stimulation when the ipsilateral cavity was open to the atmosphere anteriorly.

Since vasoconstriction in the ipsilateral and contralateral cavities occurred simultaneously, and because vasoconstriction in the contralateral cavity still occurred after acute contralateral adrenalectomy, it is unlikely to be due to catecholamine release (Malm (1973)). It is also of the opinion that the contralateral response was not due to a vasoactive agent passing through the heart because the increased pale appearance of the nasal mucosa and the decrease in venous blood flow from the nose occurred in both nasal cavities about the same time.

Post-ganglionic vasoconstrictor sympathetic nerves are known to reach the nasal vasculature of the cat mainly by the Vidian nerve; subsequently the posterior nasal nerve and some also reach it via the ethmoidal branches of the ophthalmic nerve, and also by blood vessels (Wilson & Yates 1977). The occurrence of vasoconstriction in both cavities after unilateral Vidian nerve stimulation suggests that the sympathetic supply to the vasculature of the contralateral cavity arises peripherally from the Vidian nerve, probably from the sphenopalatine ganglion or the posterior nasal nerve. The posterior nasal nerve was, however, too short to apply electrodes to determine whether it contained sympathetic fibres for both nasal cavities.

Vasoconstriction in the contralateral cavity is not due to changes in arterial blood pressure because in contrast to the experiments in which the preganglionic cervical sympathetic chain was stimulated, no increase

blood pressure was observed on Vidian nerve stimulation. The finding that vasoconstriction was evoked in the sympathectomized cavity by stimulating the cervical sympathetic chain of the opposite side further suggests that each cervical sympathetic chain may convey some fibres to the opposite nasal cavity, or that fibres going to the cavity of the same side are able to send some fibres to the contralateral cavity. Since vasoconstriction in the denervated cavity is reduced by section of either the posterior nasal or ethmoidal nerve on the side of stimulation but not by section of the anterior nasal nerve on the side of denervation, it would suggest that some sympathetic fibres to the contralateral cavity arise from the innervated side. The failure to completely abolish vasoconstriction by sectioning these nerves suggests that some fibres may be conveyed to the contralateral cavity by blood vessels.

Änggård & Densert (1974) found no evidence in the nose of the cat of fluorescent sympathetic fibres crossing the midline and no effect was seen on the exchange vessels on contralateral sympathetic stimulation. This lack of contralateral response is in contrast to the rhinomanometric studies of Malm (1973) and the present findings. Änggård & Densert pointed out, however, that because of a relative difference in the degree of the vasoconstrictor response exists between the exchange and capacitance vessels in the nasal mucosa (Änggård & Edwall, 1974) a vasoconstrictor response would most likely appear in rhinomanometric measurements which mainly reflect the capacitance function of the nasal mucosa.

Frankel (1966) has also suggested that the contralateral response he observed in dogs may be due to sympathetic fibres of one side innervating a blood vessel which supplies both nasal cavities. Malm (1974) obtained some evidence of vascular connections between the nasal cavities in cats and Abe & Jackson (1972), using a radioactive micro-

sphere technique in dogs, found a collateral circulation in the contralateral nasal cavity that was 30% of the flow in the ipsilateral cavity. Änggård & Densert (1974) suggested that if a collateral circulation does exist, a leakage of adrenergic transmitter to the blood stream might explain the variance between their results and those of Malm (1973). Since in our studies the ipsilateral and contralateral responses appear simultaneously this would appear unlikely.

The present findings suggest that sympathetic fibres reach the contralateral cavity by way of the posterior nasal or ethmoidal nerves or perarterial plexus of the opposite side. From the studies of Abe & Jackson (1972), Änggård & Densert (1974) and Malm (1974) it is, however, more likely that the sympathetic fibres from these pathways innervate blood vessels which supply both nasal cavities.

## ZUSAMMENFASSUNG

Unilaterale präganglionäre Reizung des Halssympathikus bei der narkotisierten Katze hatte eine frequenzabhängige Vasokonstriktion in den beiden Nasenhöhlen zur Folge. Die Vasokonstriktion in der kontralateralen Nasenhöhle betrug zwischen 15 und 20% der Vasokonstriktion, die in der Nasenhöhle der gereizten Seite ausgelöst wurde. Ähnliche Ergebnisse wurden durch unilaterale Reizung des Nervus Vidianus erzielt. Da die in der sympathektomisierten Nasenhöhle durch Reizung des gegenüber liegenden Halssympathikus hervorgerufene Vasokonstriktion nach Durchschneidung des Nervus posterior nasalis und des Nervus ethmoidalis auf der gereizten Seite nur reduziert wird, aber nicht total verschwindet, darf davon ausgegangen werden, daß die vasokonstriktorisches Fasern zur kontralateralen Nasenhöhle entweder über diese Nerven oder die perarteriellen Geflechte ziehen. Es ist jedoch höchstwahrscheinlich, daß die in diesen Nerven verlaufenden sympathischen Fasern Blutgefäße innervieren, die die beiden Nasenhöhlen versorgen.

## REFERENCES

- Abe Y & Jackson R T 1972 The use of labelled microspheres to determine blood flow in the dog's nasal mucosa. *Ann Otol Rhinol Laryngol* 81 82.
- Änggård A & Densert O 1974 Adrenergic innervation of the nasal mucosa in cat. *Acta Otolaryngol* (Stockh) 78 232.
- Änggård A & Edwall L 1974 The effects of sympathetic nerve stimulation on the nasal mucosa. *Acta Otolaryngol* (Stockh) 78 232.

- rate and local blood content in the nasal mucosa of the cat *Acta Otolaryngol* (Stockh) 77, 131
- Bertein, P 1929 La sympathectomie péricarotidienne dans l'ozène *Zentralbl Hals- Nasen- Ohrenheilkd* 13, 323
- Eccles, R & Wilson, H 1973 The parasympathetic secretory nerves of the nose of the cat *J Physiol* (Lond) 230, 213
- 1974 The autonomic innervation of nasal blood vessels of the cat *J Physiol* (Lond) 238, 549
- Franke, F E 1966 Sympathetic control of the dog's nasal blood vessels *Proc Soc Exp Biol Med* 123, 544
- Malcomson K G 1959 The vasomotor activities of the nasal mucous membrane *J Laryngol Rhinol Laryngol* 73, 73
- Malm, L 1973 Stimulation of sympathetic nerve fibres to the nose in cats *Acta Otolaryngol* (Stockh) 75, 519
- 1974 Responses of resistance and capacitance vessels in feline nasal mucosa to vasoactive agents *Acta Otolaryngol* (Stockh) 78, 90
- Richerson H B & Seebohm, P M 1968 Nasal response to exercise *J Allergy* 41, 269
- Slome, D 1955-56 Physiology of nasal circulation *Scient Basis Med* 5, 451
- Stocksted, P & Thomsen, K A 1953 Changes in nasal cycle under stellate ganglion block *Acta Otolaryngol* (Stockh), Suppl 109, 176
- Undritz W 1929-30 Über vasomotorische Reflexe Nase *Z Hals- Nasen- Ohrenheilkd* 25, 157
- Wilson, H & Yates, M S 1975 Crossed sympathetic innervation of the cat nasal vasculature *J Physiol* (Lond) 247, 4P
- 1977 Sympathetic nerve pathways to the nasal vasculature of the cat *Acta Otolaryngol* (Stockh) press

H Wilson, M D, Ph D  
Dept of Pharmacology and Therapeutics  
New Medical Building  
P O Box 147  
Liverpool L69 3BX,  
England

## COMPARATIVE MEASUREMENTS OF THE MUCOSAL BLOOD FLOW IN THE HUMAN MAXILLARY SINUS BY PLETHYSMOGRAPHY AND BY XENON

R. Aust, L. Backlund, H. Drettner, B. Falck and B. Jung

*From the Department of Otolaryngology, the Department of Clinical Physiology  
and the Laboratory of Radiophysics, University Hospital, Uppsala, Sweden*

(Received January 29, 1977)

**Abstract** Two different methods, one plethysmographic and one where the elimination of radioactive inert gas as measured, have been used to calculate the blood flow in the mucosa of the maxillary sinus in living man. A total of 5 persons were investigated and the mean values of the blood flow in the two methods showed a close correlation. Plethysmography is more easily performed and is therefore the preferable method to be used in studies on the physiology of the maxillary sinus.

ings of the xenon elimination the blood flow in the antral mucosa could be calculated.

The two methods compared were (1) plethysmography of the mucosa of the maxillary sinus, and (2) measurements of the elimination of radioactive xenon from the maxillary sinus of living man.

### METHOD 1 PLETHYSMOGRAPHY

Physiological studies on the gas exchange in the maxillary sinus and studies on the function of the maxillary ostium have made it necessary to find methods for quantitative determination of the blood flow in the antral mucosa.

One method for measuring the blood flow in the mucosa was reported by Drettner & Aust in 1974. This was a plethysmographic method which gave quantitative measurements of the antral blood flow even if all errors of the method were not known.

To be able to evaluate the reliability of the plethysmographic method, experiments were performed in which the elimination of radioactive xenon from maxillary sinuses with artificially obstructed ostia in healthy humans was measured in the same way as Elner & Nilsen (1970) did in the middle ear. From the record-

In manometrical investigations of maxillary sinuses with occluded or partially occluded ostia a pulse synchronous pressure wave has been registered which can be produced experimentally in healthy sinuses in which the maxillary ostium has been closed with a tampon. The magnitude of the pulse wave can be measured via a calibration which is performed by recording the pressure decrease in the measuring system caused by aspiration of a known volume of air from the system and by using Boyle's law (Drettner & Aust, 1974). The height of the pulse wave reflects the blood added to the mucosa with each heart beat, it will not show the total blood flow, however.

Such a blood flow measurement can be performed by compression of the jugular veins with two fingers on each side of the neck, thus blocking most of the venous blood flow from

This investigation was supported from The Swedish Medical Research Council (Project 749).

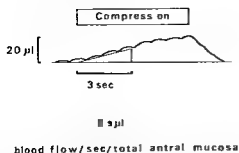


Fig 1 Plethysmography of the mucosa in the maxillary sinus during bilateral compression of the jugular veins. The maxillary ostium was blocked by a tampon in the middle meatus

the viscerocranial part of the head. During this compression the pressure in the investigated sinus rises and the slope of the pressure recording gives the increase in blood volume in the antral mucosa per second, thus showing the antral blood flow in the whole mucosa of the sinus (Fig. 1).

The volume of the maxillary sinus and the surface area of the mucosa of the maxillary sinus can be calculated according to a roentgenological method elaborated earlier (Aust & Helmius 1974) and allows us to calculate the blood flow per surface area of the antral mucosa.

We have not been able to measure the thickness of the mucosa and have therefore used the value of 125  $\mu\text{m}$ , a measure reported by Loring & Tenney (1973).

By using this value together with the results of the plethysmographic and roentgenological measurements of the maxillary mucosa and by transforming the results to a common expression for blood flow a mean value of the blood flow of the antral mucosa of 1.25  $\text{ml min}^{-1}$  and  $\text{ml}^{-1}$  mucosa was obtained in 7 subjects in a series of investigations performed in 1974 (Drettner & Aust 1974).

The reliability of the method has been questioned, however, since the jugular veins have no valves and the measured values may thus be caused only by a retrograde progression of the increase in venous pressure to the maxillary sinus. Anastomoses with other venous systems may contribute another error. To be

able to test the reliability of the relatively easily performed plethysmographic method for measuring the blood flow of the maxillary mucosa the following method was developed.

## METHOD 2

### RADIOACTIVE XENON ELIMINATION

When looking for another principle of measuring blood flow we found that measurement of the elimination of radioactive xenon has been used in studies of the mucosal blood flow in the middle ear and cell system of the mastoid process by Elner & Nilsen (1970). In these experiments quantitative calculations of the blood flow could not be performed as they could not measure the volume or surface area of the examined mucosa. In our experiments it was possible with the roentgenographic method (Aust & Helmius, 1974) to calculate the volume of the examined organ, the maxillary sinus, and the surface area of the antral mucosa. Using the thickness of the mucosa reported by Loring & Tenney (1973) the volume of the mucosa of the sinus could also be estimated.

A NaI(Tl) crystal detector with a recording unit was placed in front of the sinus and gas elimination was recorded. In the experiments,  $^{133}\text{Xe}$  with a half life of 5.3 days was used. A gas volume corresponding to 8 ml was injected into the sinus.

It was assumed that no diffusion of xenon into surrounding tissue took place once the blood had left the mucosa and before the blood reached the lungs, where all xenon was believed to be immediately and totally eliminated with the expired air.

Under these assumptions the following expression for the blood flow in the mucosa was easily derived:

$$\frac{V_s \ln 2}{V_M T_1 K} \text{ ml/min ml}$$

where

$V_s$  = sinus volume (ml)

$V_M$  = mucosal volume (ml)

- $t$  = the time to get half of the initial activity (min)
- $\lambda$  = partition coefficient, the distribution of xenon between gaseous state and soft tissue

## MATERIAL

In the comparative study on the methods for measurement of the blood flow in the mucosa of the maxillary sinus, 5 healthy persons (3 women and 2 men), ages 27 to 48, were investigated. Another person had to be discounted because of difficulties in occluding his ostium. Further, one experiment was performed on a cadaver.

### Procedure of the experiments

In the healthy subjects used in the investigation the experiments were initiated by occluding the ostium with a tampon moistened with eucortin ointment®.

After the experimental closure of the ostium a cannula connected to an electromanometer with an amplifier and a recorder was introduced into the maxillary sinus through the lower nasal meatus and was kept there throughout the experiment.

When the closure of the ostium was adequate, a pulse wave was registered in the antrum. Insufficient closure was revealed by respiratory variations in the pressure recording, which necessitated a better closure with the tampon.

The first part of the experiment was a plethysmographic measurement of the blood flow of the antral mucosa. A bilateral blocking of the venous blood flow in the internal and external jugular veins by digital pressure on both sides of the neck as earlier described was performed and the mean value of these measurements was used. Calibration by pressure recording during aspiration of a known small volume from the maxillary sinus was performed and the volume of the measuring system was measured in a similar procedure.

Table I Results of blood flow measurements ( $\text{ml} \cdot 100 \text{ cm}^{-3} \text{ min}^{-1}$ ) of the antral mucosa by plethysmography (twice) and  $^{133}\text{Xe}$  in five subjects

Case no	I	II	III	IV	V	Mean
Plethysmography I	61	115	46	122	98	88
Xenon	91	106	40	104	124	93
Plethysmography II	130	127	71	57	53	87

The measurements were performed with the subject in a semi-recumbent position.

The second part of the experiment was started by placing the subject in a semi-recumbent position with fixed head and the NaJ-crystal detector in optimal position in front of the investigated sinus.

One ml xenon containing eight  $\mu\text{Ci}$   $^{133}\text{Xe}$  was introduced into the sinus through the cannula which was thereafter blocked. The elimination of the xenon was registered with the NaJ-crystal detector and this crystal was connected to a scaler and a semi-logarithmic recorder. The recording took one to two hours.

After the measurement of the xenon elimination the investigated sinus was washed out with air, after which a new plethysmography was obtained in the same way as before the radioactive measurement. Each person in the experiment was radiographically investigated for determination of antral volume and mucosal surface area.

## RESULTS

As shown in Table I the plethysmographic measurements prior to the xenon experiments gave a mean blood flow in the antral mucosa of  $0.88 \text{ ml} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$  mucosa and, after the radioactive gas experiment, a mean blood flow of  $0.87 \text{ ml} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$ . The mean blood flow in the mucosa of maxillary sinus calculated from the measurements of the elimination of radioactive xenon from the antrum gave a mean value of  $0.93 \text{ ml} \cdot \text{min}^{-1} \cdot \text{ml}^{-1}$  mucosal tissue.

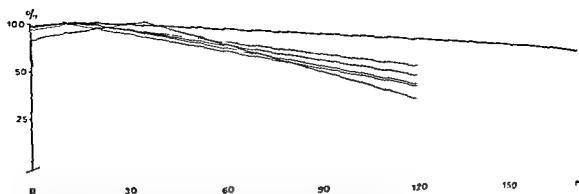


Fig 2 Recording of radioactivity from the maxillary sinus after introduction of  $^{133}\text{Xe}$  in the sinus with a tampon occluding the ostium. Values obtained in 5 subjects. The

interrupted line illustrates the mean value of the investigated subjects

As seen in Table I there were variations in the three measurements performed in each person but the results were of the same magnitude. The variations, especially in the later plethysmographic measurements, were probably due to leakage and/or irritation. As seen in Fig 2, the recordings of the elimination of radioactive xenon started with a slight increase in activity and a decrease was recorded first after about 15–20 minutes.

#### Experiments for testing the method

The background to the initial increase of activity was investigated separately. It was found to be due to a slow distribution of gas in the maxillary sinus. A similar phenomenon was reported by Elner & Nilsen (1970) in their experiments in the middle ear.

A test experiment was performed with a vertically placed glass tube, closed at both ends, with a length of 200 mm and a diameter of 15 mm in which 1 ml of radioactive xenon was injected in the bottom of the tube. A crystal detector was placed over the top of the glass tube, and the experiment showed that the gas introduced at the lower end of the tube required 40 minutes to reach a constant level at the NaI detector. The density of Xe gas is  $5.30 \text{ kg/m}^3$  and air  $1.17 \text{ kg/m}^3$  at  $30^\circ\text{C}$  and  $101.1 \text{ kPa}$ .

The same increase in activity was recorded in a cadaver experiment in the maxillary sinus with its ostium blocked with paraffin. This increase took place during the first 40 minutes. After that the radioactivity was most constant (Fig 3).

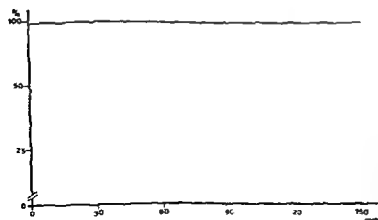


Fig 3 Recording of radioactivity in the maxillary sinus in a cadaver. Experimentally occluded ostium.

## DISCUSSION

From these last two experiments it has been shown that the distribution of radioactive xenon in an air filled, closed space is slow and that there is no elimination of xenon from an occluded maxillary sinus lacking all blood flow in the antral wall. There is of course diffusion of gas into the walls, but the solubility of xenon in tissue is very low (six times lower than that of nitrogen) and this diffusion can only have a marginal effect on the measurements.

The two methods presented for measuring the blood flow of the mucosa of the maxillary sinus are the first two methods ever presented for quantitative determination of the blood flow in any mucosal membrane in living man. This has been made possible by our methods for calculating the volume of the sinus and the mucosal surface area. The only parameter which is not known in the two presented methods is the thickness of the mucosa. However if the thickness of the mucosa is assumed to be constant throughout the experiment it will not cause any error when the blood flow is calculated from the same surface area in both methods. If the thickness is assumed to be 125  $\mu\text{m}$ , the mean antral mucosal blood flows measured with plethysmography in the 5 investigated subjects were, before and after the xenon experiments, 1188  $\text{ml min}^{-1} \text{ml}^{-1}$  and 087  $\text{ml min}^{-1} \text{ml}^{-1}$  respectively. The blood flow calculated from the elimination of  $^{133}\text{Xe}$  from the maxillary sinus was 093  $\text{ml min}^{-1} \text{ml}^{-1}$ .

A close correlation was found between the results of the two methods for blood flow measurement in the mucosa. The methods attack the problem from two entirely different angles. Since the results were of similar magnitude with the two methods it seems likely that both methods can be used for measurement of the mucosal blood flow in the maxillary sinus. The plethysmographic method is experimentally easier and does not involve any

radioactive irradiation, which facts render it the first method of choice. The variations in the values are probably principally due to the difficulty in achieving a reliable occlusion of the ostium lasting as long as required when the xenon measurements are included. The effectiveness of the vein compression is another factor which can vary.

The measurements gave rather high values of blood flow in the antral mucosa. The same magnitude of blood flow has been reported in the nasal mucosa of cat by Malm (1974), 130  $\text{ml per g nasal mucosa and minute}$ , and by Anggård (1974) 120  $\text{ml}$ . Different techniques were used in these two studies and none was similar to those described in this paper.

## ZUSAMMENFASSUNG

Zur Schätzung der Mukosadurchblutung der Kieferhöhle am lebenden Menschen wurden zwei verschiedene Methoden angewandt: eine plethysmographische und eine andere mit Messung der Ausscheidung inerten radioaktiven Gases. Insgesamt 5 Personen wurden untersucht und die Durchschnittswerte der Durchblutung zeigten in der Anwendung beider Methoden gute Korrelation. Die Plethysmographie ist leichter durchzuführen und deshalb die vorzuziehende Methode für Studien der Physiologie der Kieferhöhlen.

## REFERENCES

- Aust R & Helms G 1974 Methods for measuring the volume of the maxillary sinus in living man. *Rhinology* 12: 3.
- Drettner B & Aust R 1974 Plethysmographic studies of the blood flow in the mucosa of the human maxillary sinus. *Acta Otolaryngol* (Stockh) 78: 239.
- Einer A & Nilsén R 1970 Preliminary studies of gas resorption from the middle ear. *Acta Otolaryngol* (Stockh) 70: 197.
- Loring S H & Tenney S M 1973 Gas absorption from frontal sinus. *Arch Otolaryngol* 97: 470.
- Malm L 1974 Responses of resistance and capacitance vessels in feline nasal mucosa to vasoactive agents. *Acta Otolaryngol* (Stockh) 78: 90.
- Anggård A 1974 Capillary and shunt blood flow in the nasal mucosa of the cat. *Acta Otolaryngol* (Stockh) 78: 418.
- B Drettner M D  
Dept of Otolaryngology  
Huddinge sjukhus  
S 14186 Huddinge  
Sweden



## THE ROLE OF LOCAL GAS COMPOSITION IN PATHOGENESIS OF MAXILLARY SINUS EMPYEMA

C. Carenfelt and C. Lundberg

*From the Departments of Otolaryngology Karolinska Sjukhuset and Södersjukhuset and  
the Department of Clinical Microbiology Karolinska Sjukhuset Stockholm Sweden*

(Received February 9 1977)

**Abstract** An impaired ostial function may be of importance in the pathogenesis of the maxillary sinus empyema due to changes of the antral gas composition. Oxygen is usually not demonstrable in purulent sinus secretion while carbon dioxide accumulates. In vitro pneumococci were able to create a similar gas environment provided that the gas exchange between the medium and the atmospheric air was reduced. It is suggested that heavy antral growth of facultative anaerobes such as pneumococci is related to the antral ventilation which when impeded facilitates bacterial growth.

Onset and course of bacterial infections are dependent on the virulence of the pathogenic microorganisms, the host defence and on local conditions with regard to essential nutrients and gas composition.

In purulent maxillary sinus secretions the  $pO_2$  usually was about zero and the  $pCO_2$  frequently above 13.3 kPa (100 mmHg), in contrast to the gas composition in cases with non-purulent sinusitis as previously demonstrated (Carenfelt & Lundberg 1970). Pneumococci and *H. influenzae* were most frequently isolated from the secretions.

The present work was done to evaluate if the gas composition in purulent maxillary sinus secretion has any influence on the growth of pneumococci and if the bacteria may influence the gas composition.

## MATERIAL AND METHODS

### Patients

Sinus secretion samples were aspirated through a Lichtwitz needle introduced into the inferior concha from untreated patients with symptoms of maxillary sinusitis of longer than 30 day duration. Immediately after aspiration the secretions were classified as purulent or non-purulent (Carenfelt & Lundberg, 1977). Twenty-seven secretions were designated as purulent and 16 as non-purulent. All non-purulent secretions in this study were of serous character. In all 43 secretions from 43 patients were collected and examined for bacterial growth. Twelve purulent and serous secretions were examined for  $pCO_2$ , pH and number of bacteria and inflammatory cells. The bacterial number in purulent secretions was also determined in vitro by count. Five secretions of each variety were used in air exposure experiments.

### Gas composition

The secretions were examined for  $pO_2$ ,  $pCO_2$  using an Ultra Micro Gas Analyser 113 Instrumentation Laboratory Inc. USA as previously described (Carenfelt & Lundberg 1977).

77) Before the analyses the electrode assembly was calibrated with gases of known composition, the actual atmospheric pressure and the vapour pressure of water being taken into account.

### Bacteriological methods

A sample of sinus secretion aspirated into a syringe was transferred to a transport tube containing plain agar and to bottles, one for aerobic and one for anaerobic growth, containing proteose peptone yeast extract with sodium polyanetholsulfonate obtained from the National Bacteriological Laboratory, Stockholm, Sweden. The materials were brought to the laboratory usually within 2-3 hours and always within 12 hours and cultured on blood agar plates (aerobic and anaerobic), blood agar with gentian violet (anaerobic), phenyl ethanol agar (Difco), endoagar and hematin agar plates. The plates were incubated at 37°C and read after 24 and 48 hours. The hematin agar plates were incubated in 5% CO<sub>2</sub>. The anaerobic culture bottles were read daily either until growth occurred or for 10 days after which time subculturing on anaerobic blood agar plates was performed. GasPak (BBL, Cockville, Md, USA) was used for anaerobic cultivation. Pneumococci, *H. influenzae* and aerobic bacteria were identified according to conventional methods adopted by the Department of Clinical Microbiology, Karolinska Sjukhuset. Isolated bacteria were freeze-dried and stored at -70°C for later subculturing and classification (National Bacteriological Laboratory, Stockholm). The anaerobic culture system, including the special equipment for subculturing (Belco Glas Inc., Vineland, N.J., USA) developed by the Anaerobe Laboratory, Virginia Polytechnic Institute and State University (Holdeman & Moore, 1973) was used for subculturing.

### Quantification of bacteria and inflammatory cells

The numbers of bacteria and inflammatory cells in the sinus secretion samples were esti-

mated by counting in a Burkner chamber. Bacterial numbers less than  $1 \times 10^6$  per ml and cellular numbers less than  $1 \times 10^4$  per ml could not be counted with accuracy. The viability of inflammatory cells was determined by staining with trypan blue. Smears were stained in May-Grunewald-Giemsa for differential count. The samples were examined immediately or stored at +4°C until reading took place within one hour of aspiration. In 5 purulent secretions from which pneumococci were isolated, also viable counts by plating were done. Tenfold dilutions of secretions in saline were plated on blood agar and incubated aerobically and anaerobically.

### Air exposure

The effect of air exposure on the gas composition in sinus secretions was studied by pouring 1.5 ml of the newly aspirated secretion into a glass bowl providing an air contact area of 50 cm<sup>2</sup>. The secretions were exposed to atmospheric air at a temperature of 37°C. The pO<sub>2</sub> and the pCO<sub>2</sub> of the secretions was determined after 0, 1, 3 and 5 min. The number of bacteria and inflammatory cells in these secretions was within the range given in Table I.

### In vitro studies

A bacterial suspension from an overnight culture of *Diplococcus pneumoniae* type 3 was prepared by inoculating  $2-30 \times 10^8$  bacteria per ml in a volume of 100 ml of pre-warmed fresh Todd Hewitt broth. The suspension was poured either into flasks (Erlenmeyer) with a volume of 1000 ml, thus giving a large surface/volume ratio, or into bottles having a volume of 100 ml, thus completely filled. The 100 ml bottles were tightly sealed with a rubber membrane maintained in position by screw caps. All flasks and bottles were equipped with a magnetic stirrer and incubated at a constant temperature of 37°C. The same procedure was followed in a control experiment, except that no bacteria were added to the fresh broth. Samples of the bacterial suspension were obtained by aspiration into 2 ml

Table I *Bacteria isolated in purulent and serous secretions aspirated from 43 maxillary sinuses*

No of secretions	Number of isolates				
	D pneumoniae	H influenzae	Anaerobes	Miscellaneous	No growth
Purulent 27	12	4	9	8	0
Serous 16	3	1	0	5	8

sterile syringes at 0, 2, 4 and 8 hours for analysis. One further sample was taken 24 hours after inoculation. The  $pO_2$ , the  $pCO_2$  and the pH of the bacterial suspensions were analyzed by the same method as above. The number of bacteria was estimated in a Burkler chamber. To prevent bacterial growth between sampling and calculation, a 0.1 ml formaldehyde solution 10%, was added to 0.9 ml of the bacterial suspension. Three series of 5 experiments were performed. In the first series the flasks were used with the magnetic stirrer started at incubation to provide a maximum gas exchange. In the second, the flasks were used but with the stirrer started after 2 hours of incubation. In the third series of experiments, the sealed bottles were used to ensure that no gas exchange occurred between the bacterial suspension and the atmospheric air.

## RESULTS

### Clinical studies

Bacterial growth was found in all purulent secretions and in half of the serous secretions (Table I). Pneumococci were the most frequent findings and occurred also in serous

secretions. Anaerobes, on the other hand, were isolated exclusively from the purulent secretions with a predominance of *Streptococcus intermedius* and *Peptostreptococcus anaerobius*. The bacterial number in the purulent secretions was about 100-fold that in the serous secretions, on average (Table II). The bacterial numbers determined with viable counts and in the Burkler chamber in 5 purulent secretions with growth of pneumococci were  $1.0 \times 10^9$  colony forming units/ml and  $3.4 \times 10^8$  bacteria/ml on average.

The number of inflammatory cells in the purulent secretions was about 1000-fold that in the serous secretions, on average (Table II). The proportion of viable inflammatory cells to the total number of nucleated cells determined in 10 purulent secretions averaged 73 (23-85) per cent. In smears the inflammatory cells could be classified with accuracy in 10 of 17 purulent secretions. The neutrophilic granulocytes dominated and amounted to 50-100 (90.5-100) per cent, on average.

In the purulent secretions the  $pO_2$  was low, 0-1.2 kPa (Table II), irrespective of the bacteria isolated, i.e. pneumococci, *H. influenzae* or anaerobic streptococci. In contrast the mean  $pO_2$  in the serous secretions was significantly

Table II *Number of bacteria and inflammatory cells, the  $pO_2$  (kPa), the  $pCO_2$  (kPa) and pH in 12 purulent and 11 serous sinus secretions given as mean values*

	No of bacteria	Cells	$pO_2$	$pCO_2$	pH
Purulent					
Mean	$3.7 \times 10^8$	$3.7 \times 10^6$	0.1	$\geq 11.6$	6.8
Range	$1.0 \times 10^8 - 7.7 \times 10^8$	$1.0 \times 10^5 - 9.3 \times 10^6$	0-1.2	5.4- $\geq 13.3$	6.1-7.6
Serous					
Mean	$< 2.4 \times 10^7$	$< 4.1 \times 10^5$	11.7	5.3	7.4
Range	$< 1.0 \times 10^8 - 7.3 \times 10^7$	$< 1.0 \times 10^6 - 1.8 \times 10^6$	7.3-16.2	2.8-8.8	7.2-7.6

Table III Oxygen and carbon dioxide tension, kPa, in 5 purulent and 5 serous sinus secretions before and after exposure to atmospheric air

Volume of secretion sample 1.5 ml Contact area air/secretion 5.0 cm<sup>2</sup>

	0 min		5 min	
	pO <sub>2</sub>	pCO <sub>2</sub>	pO <sub>2</sub>	pCO <sub>2</sub>
Purulent secretions				
Mean	0	≥12.2	0	≥10.8
Range		11.8–13.3		6.7–13.3
Serous secretions				
Mean	11.1	4.4	18.1	≤2.7
Range	7.3–16.2	2.8–6.4	12.1–22.1	≤1.3–4.6

antly higher, 11.7 kPa. The mean pCO<sub>2</sub> was significantly higher in the purulent secretions than in the serous secretions ( $p < 0.001$ , Student's *t* test). The mean pH in the purulent secretions was slightly acid, 6.8, whereas in the serous secretions it was slightly alkaline, 7.4.

The gas composition in 5 purulent secretions and 5 serous secretions exposed to atmospheric air is shown in Table III. In the purulent secretions with a great number of bacteria and inflammatory cells, the pCO<sub>2</sub> decreased while the oxygen tension remained unaltered, despite 5 min air exposure. In the serous secretions, on the other hand, the pO<sub>2</sub> increased and the pCO<sub>2</sub> decreased and equilibrated with the atmospheric air, or nearly so.

#### In vitro studies

In all experiments with wide communication between air and suspension, no exponential

Table IV Gas tension, pH and bacterial number in bacterial suspension kept in open flasks at 37°C with the stirrer started at the time of inoculation

The figures are means of 5 experiments

Inoculation (h)	pO <sub>2</sub> (kPa)	pCO <sub>2</sub> (kPa)	pH	No. of bacteria/ml
0	15.8	≤1.3	7.8	1.7 × 10 <sup>7</sup>
1	13.9	≤1.3	7.9	—
2	15.0	≤1.3	7.9	4.1 × 10 <sup>7</sup>
3	16.6	≤1.3	7.9	3.4 × 10 <sup>7</sup>

growth appeared. No important alterations of neither gas composition nor pH were found (Table IV). When the magnetic stirrer was started at 2 hours, exponential growth was established in 2 of 5 experiments (Table V). Only in these two suspensions did the pO<sub>2</sub> decrease to zero and the pCO<sub>2</sub> increase during the experiment. When gas exchange was prevented between suspension and air, growth was obtained in all experiments (Table VI). The pO<sub>2</sub> decreased to zero and pCO<sub>2</sub> increased markedly after 6 hours. The gas composition in control experiments without bacteria was not changed.

## DISCUSSION

The common pathogens in maxillary sinusitis, pneumococci and *H. influenzae* are believed to have a limited capacity to overcome the defence system of the healthy mucosa. The

Table V Gas tension, pH and bacterial number in bacterial suspension kept in open flasks at 37°C with the stirrer started 2 hours after the inoculation

The figures are means of 2 experiments

Inoculation (h)	pO <sub>2</sub> (kPa)	pCO <sub>2</sub> (kPa)	pH	No. of bacteria/ml
0	14.9	≤1.3	7.7	2.7 × 10 <sup>6</sup>
2	2.3	≤1.3	7.8	—
4	0	2.7	7.1	1.1 × 10 <sup>6</sup>
6	0.7	2.8	6.8	5.4 × 10 <sup>6</sup>

Table VI Gas tension, pH and bacterial number in bacterial suspension kept in closed bottles at 37°C

The figures are means of 5 experiments

Incubation (h)	pO <sub>2</sub> (kPa)	pCO <sub>2</sub> (kPa)	pH	No of bacteria/ml
0	16.2	≤1.3	7.7	6.0 × 10 <sup>4</sup>
2	6.9	≤1.3	7.6	3.1 × 10 <sup>7</sup>
4	1.3	4.5	6.9	2.8 × 10 <sup>8</sup>
6	0	≥11.3	6.2	5.5 × 10 <sup>8</sup>

bacterial infection has to be preceded by a virus infection or other conditions causing destruction of the mucosal surface cells, tissue oedema and exudate formation. Secretion, mainly serous, is produced (van Nostrand & Goodman, 1976) and retained by the occluded ostium. The damaged mucosa and the secretion are considered favourable for bacterial colonization. The gas composition, the essential nutrients and the capacity of the local defence system at the site of the infection are important factors for the onset and course of bacterial infections. Another important factor may be the ability of the bacteria to influence the environment.

A technical procedure used for the determination of the pO<sub>2</sub> and pCO<sub>2</sub> in the sinus secretion could be expected to cause an undue admixture of air in the sample. However, in a previous study (Carenfelt & Lundberg, 1977) the method was shown to give reliable results, and in this study it was further demonstrated that any ventilation of the purulent secretions can be disregarded, as changes in the gas composition of the secretions are slow even when the contact area between atmospheric air and secretion is large.

The oxygen tension in antral air is mainly dependent on the patency of the ostium but also on the oxygen absorption by the mucosa (Aust, 1974). In retained purulent sinus secretion the gas composition may also be influenced by inflammatory cells and bacteria. In the present study the low oxygen and high carbon dioxide tension in the purulent secre-

tion were concomitant with large number inflammatory cells, mainly granulocytes, many of them viable as judged from their ability to resist staining in trypan blue. The granulocytes are not dependent on oxygen for ingestion and utilizes oxygen for certain bactericidal processes (Nathan & Bachner, 1971). In the purulent secretions with growth of anaerobic bacteria the anaerobic gas environment may be due to the influence of inflammatory cells. In a purulent secretion, however, pneumococci in pure culture were isolated. The bacteria were numerous and exceeded about 100-fold bacteria in the serous secretions. The pneumococci, which are closely related biochemically to the anaerobes, do not utilize oxygen in their energy-yielding metabolism but in the synthesis of hydrogen peroxide (Austrian, 1974).

Frequently bacteria are not only able to move oxygen from the medium, but have the ability to move it in order to make the environment suitable for their metabolism (Hewitt, 1950). The growth of pneumococci may be retarded in the presence of oxygen and facilitated in anaerobic conditions and by addition of carbon dioxide (Cowan & Steel, 1974). The *in vitro* experiments in the present study illustrate that the pneumococci were able to create a gas environment suitable for their growth and similar to that found in purulent sinus secretion, provided that the exchange with the atmospheric air was not impeded. The experiments also show that the exponential growth of pneumococci did not occur when the gas exchange was efficient.

Flottes et al (1960) suggested that altered gas composition in the sinus may be of importance to the onset of sinusitis. This hypothesis gained support when Aust & Drettner (1971) found that reduced oxygen tension in antral air is associated with ostial dysfunction. Frederick & Braude (1974) claimed that anaerobic bacterial infection in chronic sinus suppuration may be due to poor drainage and reduced antral oxygen tension.

The present results further support the opinion that an impaired antral ventilation

essential for the pathogenesis of the purulent sinusitis. It can be suggested that the impaired ventilation due to ostial occlusion facilitates the bacterial and the cellular influence on the sinus environment in such a way that heavy growth of facultative anaerobes such as pneumococci and of anaerobic bacteria is initiated and maintained. The serous sinusitis changed into the more serious purulent sinusitis.

### ACKNOWLEDGEMENT

The authors wish to thank Prof. Carl Enk Nord for his generous help with subculturing and identification of anaerobic bacteria.

### ZUSAMMENFASSUNG

Die beträchtliche Funktion des Ostium kann bei der Pathogenese von eitriger Sinusitis maxillaris von Bedeutung sein, da sich die Zusammensetzung des antralen Sinussekretes ändert. Sauerstoff ist in eitrigen Sinussekret meistens nicht vorhanden, dagegen sammelt sich Kohlendioxid an. Es gelang der Nachweis, dass *Diplococcus pneumoniae* *in vitro* eine ähnliche Gasumgebung zu schaffen imstande ist, falls der Gasaustausch zwischen dem Medium und der atmosphärischen Luft reduziert wurde. Wir schlagen vor, dass starker antraler Zuwachs fakultativ anaerober Bakterien, beispielsweise pneumococci, von der antralen Ventilation abhängig ist. Wenn diese behindert ist, wird der Bakterienzuwachs erhöht.

### REFERENCES

- Aust R 1974 Oxygen exchange in the human maxillary sinus. *Acta Universitatis Upsalensis* (Abstracts of Uppsala Dissertations from the Faculty of Medicine) 198.
- Aust R & Drettner B 1974 Oxygen tension in the human maxillary sinus under normal and pathological conditions. *Acta Otolaryngol* (Stockh) 78: 264.
- Austrian R *Manual of clinical microbiology* (ed J E Blair, E H Lennette & J P Truant). Williams & Wilkins, Baltimore 1970.
- Carenfelt C & Lundberg C 1977 Purulent and non-purulent maxillary sinus secretions with respect to  $pO_2$ ,  $pCO_2$  and pH. *Acta Otolaryngol* (Stockh) 84: 138.
- Cowan F P & Steel K J 1974 *Manual for the identification of medical bacteria*. Cambridge University Press, London.
- Flottes L, Clerc P, Riu R & Devilla F 1960 *La physiologie des sinus*. Librairie Arnette, Paris.
- Frederick J & Braude A I 1974 Anaerobic infection of the paranasal sinuses. *N Engl J Med* 290: 135.
- Hewitt L F *Oxidation-reduction potentials in bacteriology and biochemistry*. Livingstone, Edinburgh 1950.
- Holdeman L V & Moore W E C 1973 *Anaerobe Laboratory Manual*. Virginia Polytechnic Inst. and State University, Blacksburg, Va.
- Nathan D G & Baehner R L 1971 Disorders of phagocytic cell function. *Progr Hematol* 7: 235.
- van Nostrand A W P & Goodman W S 1976 Pathologic aspects of mucosal lesions of the maxillary sinus. *Otolaryngol Clin N Am* 9: 21.
- C. Carenfelt M.D.  
Dept of Otolaryngology  
Karolinska sjukhuset  
S-10401 Stockholm  
Sweden

## EXPERIENCE WITH PREOPERATIVE IRRADIATION IN HEAD AND NECK CANCER

A. E. Kortekangas, E. M. Nordman and A. Voutilainen

*From the Departments of Otolaryngology and Radiotherapy, University Central Hospital, Turku, Finland*

(Received February 20, 1977)

**Abstract** Experience with 47 patients with head and neck carcinomas treated with preoperative irradiation.

of the cases at operative biopsy. The remaining neoplastic cells were considered to be degenerated and non vital in 14% but in 68% of the cases the histological examination revealed a viable neoplasm. Changes in the macroscopic appearance did not correlate very well with the histological findings. Disadvantages of the irradiation were so minimal that the authors consider the general application of preoperative radiation in the therapy of head and neck malignancies to be the treatment of choice.

Preoperative irradiation is gaining increasing interest in the treatment of head and neck carcinomas (e.g. MacComb & Fletcher, 1957, Powers & Ogura, 1965, Strong et al., 1966, Biller & Ogura, 1970, Goldman et al., 1970, 1972, Moore et al., 1972, Virag et al., 1976). The main purpose of preoperative radiotherapy is to render neoplastic cells less capable of invading the surrounding tissues and incapable of implantation if they become distributed in the blood or lymphatic circulation during surgical extirpation.

Recommendations for the dose of preoperative irradiation and for the interval before operation have varied considerably. The volume of the irradiated area has also varied from irradiation of the manifest tumour only to an elective irradiation which includes the regional lymphatics.

It is well known that head and neck carcinomas relatively rarely and late metastasize into the lymph nodes situated outside the neck region. This fortunate behaviour has been a kind of challenge for the curative treatment of malignancies of this area and has led to routine application of neck dissection.

The recording of our data was planned to observe the effect of preoperative radiotherapy in relation to (1) the size of the tumour, (2) the microscopic characteristics of the carcinoma, (3) the operability and complications, (4) the effect on cure or failure in the treatment of malignant disease with at least 3 years observation period.

### MATERIAL AND METHODS

All 47 subjects of this series had a histologically verified head and neck region neoplasm. There was no intentional selection of patients for this type of therapy, but preoperative irradiation was the method of choice in cases of head and neck carcinomas treated in the years 1967 to 1972 at the University Central Hospital of Turku. The Radiotherapy Clinic was responsible for irradiation and the ENT Department for the diagnosis, surgical treatment and follow up observation of the patients.

Clinical patient data grouped according to the site of the tumour are given in Table 1.

the histological types of the neoplasms are given in Table II

Preoperative irradiation was delivered with Cobalt unit after an individual treatment plan to the primary tumour and included the nearest lymph nodes, usually the ipsilateral sub-digastric and mid jugular nodes. The radiation dose was from 1750 to 6000 rad, over 4-8 weeks, usually 3000 rad in 3 weeks. In most cases the preoperative dose was higher than 3000 rad. In 2 cases the radiation treatment also included the contralateral lymph nodes.

The evaluation of the effect of the treatment on the size of the tumour was estimated by careful clinical examination which was frequently repeated and often recorded by photography and measuring. The other judgements were, as a rule, confirmed by biopsy specimens.

The surgical procedure was performed 1-3 weeks after the preoperative radiation. In 2 cases the surgeon considered his extirpation to be only palliative. In 19 cases the surgery comprised a radical neck dissection, in 13 of these lymph node metastases were detected histologically without serial section investigations. In 8 cases additional postoperative irradiation was applied to a total dose of 5000-6000 rad. The observation period was at least 3 years calculated from the beginning of the preoperative radiotherapy.

## RESULTS

### 1 Effect on size of tumour

The changes in the size of the primary tumour estimated by clinical observation are given in Table III. Disappearance of the tumour was determined by inspection and palpation. In cases of disappearance the operation was carried out according to the findings before irradiation. The diminution in size was estimated in two classes: it was termed *greatly diminished* when the size at the time of operation was less than half of the original size. A *conspicuous diminution* to a size still more

Table I Location of the primary tumours and the age of the patients when the diagnosis was established

	Age limits (years)	Mean age	No of patients
Larynx	37-72	58.0	21
Oral cavity	41-74	62.1	17
Nasal cavity and paranasal sinuses	32-71	57.2	6
Hypopharynx	57-60	58.5	2
Parotid gland	38	38.0	1
Total	32-74	59.0	47

than half of the original size was termed *significant*. In 2 patients, both supraglottic laryngeal carcinomas, the size of the tumour apparently grew during the radiation therapy, and was evaluated clearly larger in the operative specimen than before therapy.

### 2 Microscopic findings

Surgical specimens for microscopy were available from 44 cases. The microscopic findings are in Table IV in relation to the TMN class of each case. In 8 cases no neoplastic cells were seen in the preoperatively irradiated tumour specimen. In 6 specimens there were neoplastic cells which were considered non-vital. Among these 14 patients in which the irradiation apparently had destroyed the neoplasm, as judged by microscopic findings, there were 3 cases in which by clinical judgement a persistent residual tumour was still present although diminished in size in 2 and unchanged in one case.

In 30 cases (67%) the histological examina-

Table II Histology of the primary tumour

Epidermoid carcinoma	
well differentiated	26
moderately differentiated	13
poorly differentiated	2
Lymphoepithelial carcinoma	1
Adenoid cystic carcinoma	3
Others (ameloblastoma, melanoma)	2
Total	47



Table III *The effect of preoperative irradiation on the size of the tumour (estimated by clinical evaluation at the operation)*

Tumour state at time of operation	The size of the primary tumour				Totals
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	
Disappeared	3	4	1	—	8
Greatly diminished	—	5	—	—	5
Significantly diminished	2	2	4	1	9
Unchanged	1	10	10	2	23
Enlarged	—	—	1	1	2
Totals	6	21	16	4	47

tion revealed viable malignant cells in the operative specimen after preoperative irradiation. Among these cases there were 2 in which—by clinical evaluation—the tumour seemed to have disappeared totally. Unfortunately no histological examination of the surgical specimen was made in 3 cases.

In the 41 cases of epidermoid carcinoma the effect of radiation on the size of the tumour was correlated to the histological differentiation degree (Table V). Moderately or poorly differentiated neoplasms did not diminish significantly more often (9/15) than the well differentiated ones (11/26) ( $\chi^2=0.76$ ,  $df=1$ ,  $p=0.61$ ).

### 3 Operability and untoward effects

The effect of the preoperative irradiation upon operability was evaluated immediately before the operation in 22 cases, a better operability was recorded for 5 cases and no apparent change in 17 cases compared with the stage at the start of the preoperative therapy. In the remaining 25 cases the surgeons' opinions of the effect on operability were vague but no cases with apparent deterioration of operability were recorded.

The surgeons considered in 18 cases that an increase of operative difficulties could be attributed to the preoperative irradiation. The most common disturbance was excess bleeding. In half the cases radiation oedema was considered to interfere with extirpation of the

Table IV *Histological findings after preoperative irradiation in operative specimens from the site of the primary tumour*

	No neoplastic cells discovered	Only devitalized neoplastic cells encountered	Vital neoplastic cells present	T
Stage I T <sub>1</sub> N <sub>0</sub>	2	1	2	
Stage II T <sub>2</sub> N <sub>0</sub>	2	2	9	1
Stage III T <sub>3</sub> N <sub>1</sub>	1	2	2	
T <sub>3</sub> N <sub>0</sub>		1	8	
T <sub>3</sub> N <sub>1</sub>			5	
T <sub>3</sub> N <sub>2</sub>	1		2	
T <sub>4</sub> N <sub>1</sub>			1	
Stage IV T <sub>4</sub> N <sub>3</sub>			1	
T <sub>3</sub> N <sub>2</sub>	1			
T <sub>3</sub> N <sub>3</sub>	1			
Total	8	6	30	4

tumour. The appearance of operative or postoperative complications seemed to have no relation to the radiation dose. The preoperative radiation could be considered responsible for somewhat delayed healing in 7 cases. Fistulae developed postoperatively in 8 patients, a disturbance which can also be regarded due to preoperative irradiation.

### 4 Survival after combined therapy by preoperative irradiation and surgery

The surgeon decided in 2 cases, both in Stage III (T<sub>3</sub>N<sub>0</sub>), to make only a partial extirpation.

Table V *The relation between the differentiation degree of epidermoid carcinoma and the response to the preoperative irradiation*

Tumour at the time of the operation	Well differentiated	Poorly or moderately differentiated	T
Disappeared	7	1	1
Greatly diminished	—	1	1
Significantly diminished	4	4	1
Unchanged	15	4	1
Enlarged	—	2	1
	26	15	41

Table VI General result of the preoperative irradiation and surgery after at least 3 years follow-up in relation to the original stage of the tumour

	Recovery from the malignancy		Local recurrence appeared		Died with metastases but recovered from the primary neoplasm	Total
	Living	Dead	Living	Dead		
stage I T <sub>1</sub> N <sub>0</sub>	4	—	—	—	1	5
stage II T <sub>2</sub> N <sub>0</sub>	9	—	—	1	4*	14
stage III T <sub>3</sub> N <sub>1</sub>	4	—	—	2	—	6
T <sub>3</sub> N <sub>2</sub>	3	2 <sup>b</sup>	—	3 <sup>c</sup>	2	10
T <sub>4</sub> N <sub>1</sub>	2	—	—	3 <sup>c</sup>	—	5
T <sub>4</sub> N <sub>2</sub>	—	—	1 <sup>d</sup>	2	—	3
T <sub>4</sub> N <sub>3</sub>	—	—	—	—	1	1
stage IV T <sub>4</sub> N <sub>3</sub>	1	—	—	—	—	1
T <sub>5</sub> N <sub>3</sub>	—	—	—	—	1	1
T <sub>6</sub> N <sub>3</sub>	—	—	—	—	1	1
Total	23	2	1	11	10	47

\* The cause of death for one patient other than the malignant disease

<sup>b</sup> The patients died 2 resp. 5 years later from other disease without signs of recurrent neck neoplasm

<sup>c</sup> Both local recurrence and lymph node metastases

<sup>d</sup> The patient is still alive 9 years later and 7 years after secondary operation

the tumour in order to avoid excessive mutilation of the patient. Both of these patients died from the cancer within 2 years, irrespective of additional postoperative irradiation. In the remaining 45 cases the surgical procedure was evaluated radical by the surgeon. As Table VI reveals, a recurrence appeared within 3 years in 12 cases and metastases in 10 additional cases either regionally or distally. The recovery percentage with 3 years' follow up is thus 53% (25/47) for the whole series, 68% (13/19) for the cases of Stages I-II and 43% (12/28) for the Stages III-IV.

#### 5 Metastases during follow-up period

Appearance of metastases during the 3 year follow up period is given in Table VII. The Table shows that metastases appeared in 7 cases of stage N<sub>0</sub>. Surgical radical neck dissection was not performed in any of these cases. The only recurrence of metastases of Stage N<sub>1</sub> after surgical neck dissection appeared on the contralateral side. The failure to perform a radical neck dissection in combination with

the extirpation of the primary tumour of N<sub>1</sub> patients was due in one case to unwillingness of the patient, and a delayed bilateral neck dissection could not save another patient.

## DISCUSSION

Complete disappearance of a clinically evident tumour as a response to irradiation has often been observed. This appeared in 8 out of 47 cases of our series: all of epidermoid carcinoma. Six of these were well differentiated and 2 were anaplastic. The incidence of disappearance in our series corresponds to that in the report of Moore et al (1972) for example. On the other hand in 2 cases of our series no response at all could be observed as the tumour continued to increase in size during the preoperative radiation therapy.

When the changes in size are compared with histological findings in the operative specimens the most conspicuous finding in this series is the unreliability of the disappearance of the tumour. In 2 of the present cases the

Table VII Incidence of lymph node metastases or their recurrence within 3 years of the operative irradiation and surgery

Original N class	So far free of metastases or their recurrence		Secondary metastases appeared		Total
	No neck dissection	Neck dissection performed	No neck dissection	Neck dissection performed	
N <sub>0</sub>	20	5	7	—	32
N <sub>1</sub>	—	9	2	1*	12
N <sub>2</sub>	—	1	—	2	3
	20	15	9	3	47

\* Metastases appeared on the opposite side of the neck which was not dissected

histological examination revealed viable neoplastic cells although the clinically recognizable tumour had responded to the radiation by total disappearance. Surgery after preoperative radiation should be carried out according to the primary plan even in cases with distinct radiation response and carefully reasoned also in cases in which complete disappearance of the tumour appears as also Goldman et al (1972) and Olofsson & van Nostrand (1973) have suggested.

Surgeons often blame preoperative irradiation for additional technical difficulties during surgery. In the present series radiation of up to 4000 rad, occasionally even more did not cause any excessive difficulty during or after the operation in accordance with Jakobsson & Wersall (1973) and Virag et al (1976).

In the present series 20% of the N<sub>0</sub> cases subsequently developed regional lymph node metastases, thus indicating a probable existence of sub-clinical metastases some time before they became palpable. Radiation treatment to the regional lymph nodes to a dose of 5000 to 5500 rad in 7–8 weeks should be considered seriously in most cases for eradication of sub-clinical metastases as Fletcher (1972) has proposed. The additional effect of the preoperative irradiation cannot be calculated from the present series without a comparative group of patients not irradiated.

The survival figures however are consistent with those of Virag et al (1976)

for example, who demonstrated significant advantages of preoperative irradiation. Experience does not show any significant benefit induced by the preoperative irradiation and equally consider its favourable effect significant.

### RÉSUMÉ

L'expérience avec 47 patients souffrant du carcinome de la tête et du cou opérés après irradiation d'un voltage de 3000 rad pendant plus de 3 semaines est portée. Pour cette série la survie était 25/47 (53 cent) pour les cas du grade I–II 13/19 (68 pour cent) du grade III–IV 12/48 (43 pour cent). Par biopsy on ne pouvait pas trouver de cellules néoplasiques dans 18 pour cent. Les cellules néoplasiques étaient considérées comme dégénérées et non vitales dans 14 pour cent mais l'examen histologique a révélé un néoplasme vital dans 66 pour cent. Les changements à l'apparition macroscopique se ne rapportaient pas très bien aux lésions histologiques. Les désavantages de l'irradiation étaient tellement minimaux que les auteurs considèrent l'application générale de la radiation préopératoire dans la thérapie du cancer de la tête et du cou comme traitement de choix.

### ZUSAMMENFASSUNG

Erfahrungen mit präoperativer Megavoltbestrahlung bei otolaryngologischen Karzinomfällen werden berichtet. Die allgemeine 3 Jahres Heilungsrate betrug 51%. Die Stadien I–II jedoch 68%. Bei histologischen Untersuchungen der Operationspräparate wurden in 18% in 14% degenerierte und zerstörte Tumorzellen gefunden aber offenbar vitales Tumorgewebe gefunden. 4% der makroskopischen Befunde der Regressions- oder Gleichbleiben des Tumors nach der präoperativen Bestrahlung war es nicht möglich die histologischen Änderungen zu beurteilen. Die Autoren finden die präoperative Bestrahlung so gering daß sie eine präoperative Bestrahlung als Routinemethode in Head and Neck Karzinomen empfehlen.

## REFERENCES

- Biller H F & Ogura J H 1970 Planned preoperative irradiation for laryngeal and laryngopharyngeal carcinoma. *Front Radiation Ther Oncol* 5 100
- Fletcher J H 1972 Elective irradiation of subclinical disease in cancers of the head and neck. *Cancer* 29 1450
- Goldman J L, Gunsberg M J, Friedman W H, Ryan J R & Bloom H S 1970 Combined therapy for cancer of the laryngopharynx. *Arch Otolaryngol* 91 221
- Goldman J L, Silverstone S M, Roffman J D & Brken E A 1972 High dosage pre-operative radiation and surgery for carcinoma of the larynx and laryngopharynx. A 14 year program. *Laryngoscope* 82 1869
- Jakobsson P Å & Wersäll J 1973 Combined radiotherapy and surgery in treatment of carcinoma of the tongue. *Acta Otolaryngol* (Stockh) 75 321
- MacComb W S & Fletcher J H 1957 Planned combination of surgery and radiation in treatment of advanced primary head and neck cancers. *Am J Roentgenol* 77 397
- Moore C, Mullins F & Scott R M 1972 Preoperative irradiation in cancer of the head and neck. *Am J Surgery* 124 555
- Olofsson J & van Nostrand A W P 1973 Growth and spread of laryngeal and hypopharyngeal carcinoma with reflections on the effect of preoperative irradiation. *Acta Otolaryngol* (Stockh) Suppl 308
- Powers W E & Ogura J H 1965 Preoperative irradiation in head and neck cancer surgery. *Arch Otolaryngol* 81 153
- Strong M W, Henschke U K, Nickson J J, Frazell E L, Tollefsen H H & Hilaris B S 1966 Preoperative X ray therapy as an adjunct to radical neck dissection. *Cancer* 19 1509
- The TNM Classification of Malignant Tumours* 1968 UICC Geneva
- Vrtać M, Krayna M, Kosoković F, Kubovic M, Zunter F, Voskresensky I & Smiljanic M 1976 Die präoperative Bestrahlung von Patienten mit Larynx und Hypopharynxkarzinom. *Laryngol Rhinol* 55 477
- A E Kortekangas M D  
Dept of Otolaryngology  
University Central Hospital  
SF 20520 Turku  
Finland

# THE INFLUENCE OF CIGARETTE SMOKE ON THE PHARYNGEAL MUCOSA

Z. Radsel and V. Kambič

*From ENT Clinic University of Ljubljana Ljubljana Yugoslavia*

(Received February 22 1977)

**Abstract** The influence of cigarette smoke on the pharyngeal mucosa was investigated in a clinical study and in an experiment on animals. Histologic reports were evaluated according to the Kambič Lenart classification of hyperplastic aberrations of the throat mucosa. A close dependence of the degree of hyperplasia and the number of cigarettes smoked was revealed. The more cigarettes the patients smoked every day, the more evident were changes on the mucosa, more clearly manifested in male than in female smokers. It was observed that the changes related to the hyperplasia atypica were more evident the longer the patients smoked. They were also more intense the younger the smokers were when they started smoking. In the experiment on animals a number of factors with additionally noxious influence on the pharyngeal mucosa were excluded. A great interdependence was observed between the number of daily inhaled cigarettes and the changes on the pharyngeal mucosa.

Various pathological changes on the pharyngeal mucosa and the number of malignancies in this region are on the increase. Frequent occurrences of disease in the upper respiratory tract are associated with an altered way of life and a high standard of living and industrialization. Smoking today stands accused as a significant factor in the incidence of specific diseases. According to the reports of the WHO, 80% of all cancers are caused by exogenous agents or these agents contribute at least to some extent to the incidence of cancer (Silverberg & Holleb 1973). Kambič & Lenart (1968, 1971) believe that endogenous factors, i.e. hormones, prepare favourable group for

the activity of the exogenous agents and are involved in the increased risk of cancer of the larynx.

Research has been conducted to determine the effect of cigarette smoke on the laryngeal mucosa in humans and in experimental animals. We wanted to see whether the increased number of cigarettes smoked daily might lead to changes in the pharyngeal mucosa. These changes were evaluated by the Kambič Lenart classification of hyperplastic aberrations in the mucous membrane of the throat (Fig. 1). The authors divide hyperplastic changes into three grades—simple, abnormal, and atypical hyperplasia. Hyperplasia simplex and hyperplasia abnormalis represent mainly the hyperplastic process on the epithelium of the laryngeal mucosa which normally does not turn malignant. In a simple hyperplasia the epithelium thickens on account of the proliferative cell layer. The basal layer remains unchanged. In subepithelial tissues very few immunocompetent cells are observed. In abnormal hyperplasia, the epithelium thickens on account of the basalification. Basal cells extend to the middle of the epithelium, there are no pathologic mitoses or atypias anywhere. Subepithelial tissue contains more immunocompetent cells. In the atypical hyperplasia cells of the entire hyperplastic epithelium have the shape of basal cells. Nuclei are hyperchromatic and slightly polymorphic, here and there partially atypical mitoses appear. The subepithelial

This research has been sponsored by the Slovene Research Association.

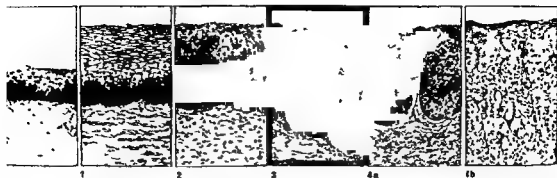


Fig. 1. The Kambič Lenart classification of hyperplastic changes on laryngeal mucosa. 0 Normal epithelium, 1,

hyperplasia simplex, 2 hyperplasia abnormalis, 3 hyperplasia atypica. 4a Ca in situ, 4b, Ca invasivum.

l tissue is full of immunocompetent cells, which is significant for this aberration.

By experimenting on animals we wanted to confirm, accept, or refute our investigations in humans.

## METHODS

Seventy-six male and 36 female patients have been surveyed. They suffered chronic tonsillitis and underwent tonsillectomy. The patients were mostly young people, aged 24, on average, except for 3 who were 16 and one who was over 50. Their occupations ranged from farm and factory workers to clerks.

In 41 patients, tonsils were removed because of frequent purulent tonsillitis. Besides recurrent purulent tonsillitis, 32 patients suffered pain in the throat, a sense of discomfort and of having a foreign body in the larynx during phases of the disease. Three patients had peritonsillar abscesses, one had polyarthritides, one had kidney inflammation, and one alopecia areata.

After the removal of tonsils, a piece of the mucosa from the left and right frontal alveolar arch was removed for histologic examination. The operation was in no way more complicated by this additional procedure, nor did the patient suffer any injury, since the tissue taken was that which is normally removed in tonsillectomy.

The patients were divided into five groups,

*ad modum* Ryan et al (1955), by the number of cigarettes they smoked per day.

The first group consisted of those who smoked up to 9 cigarettes per day. In the second group they smoked 10 to 15, in the third 16 to 20, in the fourth 21 to 34, and in the fifth, more than 35. In addition, there was a control group of non smokers.

For the inhalation of cigarette smoke in animals, a special method was developed, based on the flow of air and cigarette smoke. We used a special apparatus which enabled a constant apportionment of gases and the flow of the mixture through the container. This procedure made a longer exposure to the cigarette smoke possible. The apparatus consisted of a glass cylinder and a cover with a rubber washer on its brim and three openings through which the blend of air and cigarette smoke entered and gases from the box were released. The third opening was used for measuring the temperature, moisture, CO, and CO<sub>2</sub> in the box. Cigarettes burned in a special Raucher Vulkan's device which pulsed a mixture of air and the smoke of three cigarettes into the box through a rubber tube. Gases were pumped from the box by a water pump connected to the water network. 42 male mice of the Albany breed, 4 months old, were used in this experiment. The mice were divided into five groups of 7 and a control group which was not exposed to cigarette smoke. The smoke of three cigarettes was in

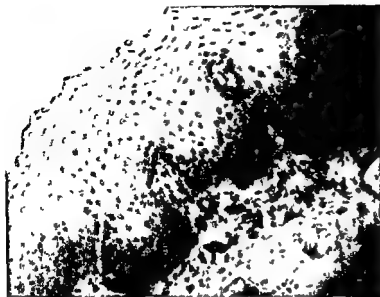


Fig 2 Pharyngeal mucosa in human  
Hyperplasia simplex HE,  $\times 235$

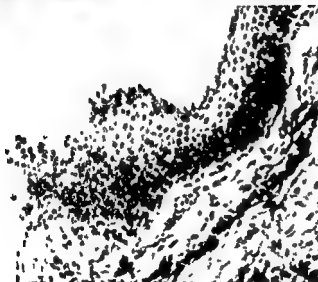


Fig 3 Pharyngeal mucosa in human  
Hyperplasia abnormalis HE  $\times 255$

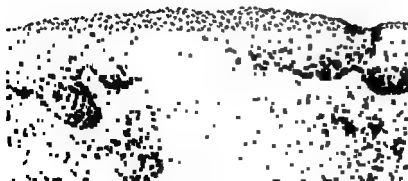


Fig 4 Pharyngeal mucosa in human  
Hyperplasia atypica HE  
 $\times 210$

Table I Relationship between the degree of hyperplasia and the number of cigarettes smoked daily

Number of cigarettes	Normal	Hyperpl simplex	Hyperpl abnormalis	Hyperpl atypica
0	3	11	1	0
9	0	4	7	4
15	0	2	10	3
20	0	1	9	4
34	0	0	8	6
40	0	1	3	5

haled by the mice once to five times per day, five times a week, during 10 weeks. The mice were sacrificed immediately after the last exposure. In one mouse from each group, femoral vein was incised, blood for the COHb test was examined, and the pharyngeal mucosa was examined microscopically.

## RESULTS

In checking the first group of patients, we observed that 4 patients had signs of simple hyperplasia (Fig 2), 7 patients showed abnormal hyperplasia (Fig 3), and 4 the atypical one (Fig 4). In the second group, 2 patients had a simple, 10 the abnormal, and 3 the atypical hyperplasia.

In the third group the simple hyperplasia was diagnosed in one patient, the abnormal in 9, and the atypical in 6. In the fourth group, 8 patients showed the abnormal, 8 the atypical hyperplasia. In the fifth group one patient showed the simple stage of hyperplasia, 3 the abnormal, and 5 the atypical one.

In 3 patients from the control group the pharyngeal mucosa was healthy. A simple hyperplasia was observed in 11 patients of this group and only in one patient was the abnormal stage of hyperplasia diagnosed (Table I).

The experimental mice were regularly

Table III COHb in mice blood

Group	COHb (%)
Control	0
1	20
2	20
3	28
4	34
5	31

weighed and the relative weight increase was measured. Their weight diminished when the daily number of the inhaled cigarettes increased. The highest relative weight increase was observed in the mice of the control group (Table II). No COHb in blood was found in animals of the control group. The COHb level increased with the number of exposures to the cigarette smoke up to 34% as in heavy smokers (Table III).

Autopsy of the mice revealed no macroscopic pathologic changes on the pharyngeal mucosa. The mucous membrane was pale pink and moist. In one mouse from groups 2, 3, 4 and 5, an atelectasis of lungs was observed. In 2 mice from group 4, changes in the lungs were observed which were histologically diagnosed as abscesses. Microscopic examination revealed that the thickness of the epithelium had decreased on account of the prickle cell layer, when the number of exposures increased. With the increased number of inhalations the number of mitoses increased. These were measured in ten fields of view (Table IV).

The following observations were made in separate groups:

1st group—a simple hyperplasia was observed in 2 mice, atypical in one, abnormal in 4.

2nd group—simple hyperplasia was seen in 2, abnormal in 4 mice, atypical in one.

3rd group—simple hyperplasia developed three times, abnormal and atypical twice each.

4th group—simple hyperplasia was seen in one mouse, abnormal in 4, atypical in 2.

Table II Relative weight increase in mice

Group	control	1	2	3	4	5
Increase	4.4	3.9	2.2	3.2	1.6	0.7





Fig 5 Pharyngeal mucosa in mouse  
Normal epithelium HE  
 $\times 255$



Fig 6 Pharyngeal mucosa in mouse  
Hyperplasia simplex HE  $\times 255$



Fig 7 Pharyngeal mucosa in mouse  
Hyperplasia abnormalis HE  $\times 255$

Table IV Number of mitoses in different groups (measured in ten fields of view)

Group	Number of mitoses
Control	9 28
1	9 28
2	14 28
3	13 71
4	13 57
5	19 00

5th group=abnormal hyperplasia developed in 3 mice, atypical in 4

Five mice in the control group had a healthy mucosa, and 2 showed symptoms of simple hyperplasia (Figs 5-7)

## DISCUSSION

In spite of the obvious influence of the cigarette smoke on the incidence of malignancies in the pharynx, it is impossible to establish its effect accurately

We are of the opinion that alcohol and hygienic and dietetic factors (Rothman & Keller, 1972; Schmidt & DeLint, 1972) are also associated with the occurrence of cancer in this region. It is very difficult to determine the effect of separate agents. Differences in their degree of risk render the evaluation of the effect of these factors even more complex. Besides, the number of the cigarettes smoked each day is not constant, all smokers do not inhale in the same way, nor exhale the same composition of gases.

In the experiment on animals these factors were not considered, and consequently only the effect of the cigarette smoke on the pharyngeal mucosa could be investigated.

The basic prerequisite for the experiment on animals was the production of a human model of smoking, though it was not possible to develop an ideal one.

In smokers, solid particles of the cigarette smoke are arranged differently, the bulk of them remaining in the nose (Dalhamn et al., 1968). In humans, lungs are the most exposed

region, in animals, fewer particles enter the lungs. Almost any method of supplying cigarette smoke to animals is unnatural and even with the best technical equipment, the dose of cigarette smoke which the animal receives cannot be exactly established (Armstrong et al., 1974).

We have proved, however, that cigarette smoke has a harmful effect on the pharyngeal mucosa of mice. The greater was the number of cigarettes inhaled, the more severe were the changes on the mucous membrane ( $r=0.70$ ,  $\alpha=0.001$ ). With the increase in daily number inhaled cigarettes, the number of mitoses rose ( $r=0.50$ ,  $\alpha=0.001$ ), the epithelium grew thinner on account of the prickle cell layer and the basal layer grew.

In the experiment on animals, a number of factors having an additionally noxious effect in humans, were eliminated, and consequently, our observations show only the influence of the cigarette smoke. We have to consider the fact that exposures were of short duration, that the animals were sacrificed immediately after the experiment and that the corneous mucosa of the pharynx in mice is less prone to the harmful effect of smoking than is so in humans. However, there is evidence that even in animals the cigarette smoke does affect the pharyngeal mucosa.

## CONCLUSION

In our research into the dependency between the degree of hyperplasia and the number of

Table V Relationship between the degree of hyperplasia and the number of cigarettes inhaled daily in mice

Number of cigarettes	Normal	Hyperpl. simplex	Hyperpl. abnormals	Hyperpl. atypica
0	5	2		
3		2	4	1
6		2	4	1
9		3	2	2
12		1	4	2
15			3	4

cigarettes smoked daily, the following conclusions were reached

The greater was the number of cigarettes smoked, the greater were the changes on the pharyngeal mucosa ( $r=0.75$ ,  $\alpha=0.001$ ). The earlier the age when smoking started, the greater the changes that occurred ( $r=0.51$ ,  $\alpha=0.001$ ). Our studies indicate that in male smokers, smoking the same number of cigarettes daily, the changes that fall into the atypical hyperplasia category were more frequent than in women.  $\chi^2$  test for evaluating the relationship between the variables has proved significant at the 0.01 level.

Differences in the degree of hyperplasia show that there are endogenous agents to be considered, as has already been pointed out by Kambič et al. (1973).

## ZUSAMMENFASSUNG

Der Einfluß des Zigarettenrauches auf die Rachenschleimhaut wurde in einem klinischen Experiment und im Tierversuch untersucht. Die histologischen Resultate wurden nach der Klassifikation von Kambič und Lenart für die hyperplastischen Aberrationen an der Kehlkopf Schleimhaut gegliedert. Die Autoren haben einen großen Zusammenhang zwischen dem Schweregrad der Hyperplasie und der Zahl täglich gerauchter Zigaretten gefunden.

Je mehr Zigaretten die Patienten täglich rauchten, so größer waren die Veränderungen an der Rachenschleimhaut ausgeprägter beim Mann als bei der Frau. Der Grad der Hyperplasie war auch von der Zeit abhängig: je länger die Patienten rauchten, desto mehr atypische Hyperplasien wurden in den histologischen Präparaten gefunden. Im Tierversuch konnten einige zusätzliche schädliche Faktoren für die Rachenschleim-

grad der Hyperplasie der Rachen Schleimhaut

## REFERENCES

- Armitage A K, Houseman T H, Turner M D, Wilson D A 1974 The evaluation of a method introducing tobacco smoke into the lungs of anesthetised animals during spontaneous respiration. *Q J Exp Physiol* 59: 43.
- Dalhamn T, Edmonds M L & Rylander R 1974 Retention of cigarette smoke components in the lungs. *Arch Environ Health* 17: 746.
- Kambič V & Lenart I 1968 Untersuchungen über Wirkung von Testosteron auf die Kehlkopf Schleimhaut des Hundes. *HNO* 11: 327.
- 1971 Notre classification des hyperplasies de l'épithélium du larynx au point de vue pronostic. *J Fr Otolaryngol* 20: 1145.
- Kambič V, Lenart I, Lenart V & Radšiel Z 1973 Beitrag zur Frage der Reversibilität bzw. Irreversibilität von Gewebsveränderungen die bei Hunden der im Verabreichung von Testosteron an laryngealen Schleimhaut auftreten. *HNO* 21: 300.
- Rothman K & Keller A 1972 The effect of exposure to alcohol and tobacco on risk of cancer of the mouth and pharynx. *J Chron Dis* 25: 711.
- Ryan R F, McDonald J R & Devine K D 1971 The pathologic effect of smoking on the larynx. *Pathol* 60: 472.
- Silverberg E & Hoffer A I 1973 Cancer of the larynx. *Cancer* 32: 1177.

Z. Radšiel MD  
Otorinolaringološka klinika  
Klinični center  
Zalaska 2  
61000 Ljubljana  
Yugoslavia

## THE EARLY VASCULARIZATION OF AN AUTOGENOUS BONE INLAY INTO AN ARTIFICIAL DEFECT IN THE RABBIT MANDIBULA

A Nathanson

*From the Department of Otolaryngology, Karolinska Sjukhuset, Stockholm. The Surgical Research Laboratory, Thoracic Clinics, Karolinska Sjukhuset, Stockholm and the King Gustaf V Research Institute, Karolinska Institutet, Stockholm, Sweden*

(Received January 17 1977)

**Abstract** The revascularization of two different autogenous bone grafts to an artificial defect in the rabbit mandibula was studied by means of microangiography using Indian ink infusion into the carotid artery. The revascularization was compared between a longitudinally split autogenous humeral full thickness bone graft in 19 rabbits and autogenous full bone grafts from the iliac crest in 14 rabbits. The vascular pattern and ingrowth was followed at weekly intervals right to the end of the first month postoperatively. A progressive revascularization was found in both grafts investigated. The periosteum of the humeral graft was earlier and more completely revascularized than when an iliac graft was used. The penetration and downgrowth of capillaries were on the other hand more complete within the iliac graft. The vascular pattern is discussed with special reference to the osteogenesis in the bone grafts.

a favourable bone growth. Stringa (1957) claimed that "there is a striking correlation between the rate of vascular penetration of the bone implants and their ultimate "take" or incorporation in the bed". Zuckman et al (1968) believed that the development of newly formed vessels is a necessary preliminary stage for osteogenesis but not sufficient in itself.

The purpose of this investigation was to study the early vascularization of a split full thickness autogenous humeral respectively autogenous full thickness iliac bone graft into an artificial defect in the rabbit mandibula.

The role of the vascularization as regards the osteogenesis of bone grafts has been debated in clinical as well as in experimental studies of bone transplantation. Phemister showed in his basic experimental work (1914) that the viability of the cells of the transplant is largely dependent upon their ability to get nutrition. He also claimed that the periosteum and the endosteum superficially located received sufficient nutrition to survive and proliferate. Albee (1921) stated that an early and adequate blood supply to the graft is necessary for

### MATERIAL AND METHODS

Fifty two young adult rabbits about 6 months of different breed and sex were operated on. The operations were carried out with the animals under general anesthesia maintained by intravenous injections of Mebumal® (ACO) in a dose of approximately 30-35 mg per kg body weight and inhalation of Ether ad narcosis® (Skånska Bomullskrut). Meticulous aseptic precautions were taken. The right mandibula was exposed through a curved incision along the inferior border of the jaw bone and a 15×5 mm segment of the bone was cut out using a

This work was supported by grants from the Karolinska Institutet (grant no. 422-6) and Cancerföreningen (grants 74 36 and 74 77).

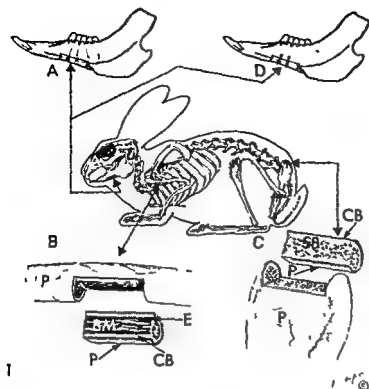


Fig 1 Operative procedure (A) Defect in the lower border of the mandible. Dotted line indicates the mandibular angle. (B) Longitudinally split full bone graft from the humerus with perosteum (P), cortical bone (CB), endosteum (E) and bone marrow (BM). (C) Full bone graft from the iliac crest with perosteum (P), thin cortical bone (CB) and spongy bone (SB). (D) Bone inlay (from the humerus or the crest) secured to the host bone by osteosutures.

0.3 mm wide diamond saw-blade (Fig 1) by means of a motor-driven drill (MEDA) under continuous irrigation with sterile Ringer's solution. The resected piece of bone and periosteum included the content of the mandibular canal and the apical part of the three first molar teeth with an extension from the mandibular angle to the symphysis of the mandible. In one group of 23 rabbits, a full bone autograft from the iliac crest was placed as an inlay transplant into the mandibular defect. The transplant included periosteum with a thin shell of cortical bone surrounding the cancellous bone of the crest. In the other group consisting of 29 rabbits, an autograft from the right diaphysis of the humerus was split longitudinally and placed together with its periosteum, endosteum and bone marrow into the defect in the jaw bone. The purpose of shaping as large a graft as possible without hand-capping the animals resulted in a humeral graft about 1 mm shorter in height than the graft from the iliac crest. The fresh bone grafts were never kept *ex corpore* for more than 20

minutes and were during the preparation two bore-holes in the host bone for the sutures kept in physiological saline solution. The transplants were then secured to the host bone by means of two 0.3 mm wide stainless steel osteosutures passing through bore holes in the mandibula and around the transplant. The incised jaw muscles and the skin were closed with interrupted sutures. The wound was then dressed with an adhesive solution of Neobucutan® (Bofors). All animals received injections of penicillin 1.1 million IE immediately after the operation and on the two succeeding days. The animals were fed on 2-3 dl per day of food pellets (Fors) with a standardized content of calories, minerals and vitamins. The rabbits were sacrificed at weekly intervals ranging from 9-30 days postoperatively.

## METHODS OF INVESTIGATION

### Microangiography

A microangiographic technique with India ink infusion was used in the study of the

vascularization of the graft. On the day of sacrifice the animals received Heparin\* (Vit-n) 10000 IE, 5 ml Xylocain\* (Astra) 0.5% and an overdose of Mebumal\* (ACO) intramuscularly. Thereafter the right common carotid artery was exposed and cannulated in cephal direction and 300–400 ml of a 20% Indian ink solution was infused during 1–1½ hours from a height of 150 cm above the heart level of the animal, as described by Olerud & Backwardt Lilliestrom (1971).

#### Preparation of specimens for morphological analyses

After the completion of the Indian ink infusion the skull was removed and the jaw bone was freed from soft tissues except for a thin periosteal layer surrounding the bone. The two osteosutures were carefully extracted. The transplant and surrounding host bone were transversally divided into three equal parts by a specially designed saw with a 3 mm diamond blade under continuous irrigation and under adjustable pressure. One part was prepared for light microscopy, one for fluorescence microscopy and one for microangiography according to the Spalteholz technique. The parts investigated with these techniques were chosen at random and varied individually so that one piece of a specimen could be investigated by three different methods.

#### Preparation for light microscopy

The specimen was fixed with a solution of formaldehyde 10% for 2 days, decalcified with trichloroacetic acid 3.5% for one week, embedded in paraffin wax and cross sectioned in 10 µm thick slices with a sliding microtome (MSE). Alternating sections were stained with hematoxylin and eosin, and Mallory's Azan method. A macrophotograph of the section was taken with a Hasselblad camera adapted for Bellows and Zeiss lummar lenses. The observations of histological sections were performed in ordinary light microscopy and photographed with a Zeiss photomicroscope.

#### Preparation for microangiography

After fixation with 10% formaldehyde solution and decalcification with trichloroacetic acid 3.5%, the specimen was embedded in paraffin wax and cross sectioned with a diamond saw blade using the above mentioned specially designed saw under continuous irrigation and adjustable pressure into 5–1 mm thick slices, deparaffinized in xylol, and clarified with methylsalicylate according to the technique described by Spalteholz (1914). The Spalteholz preparations were examined in a stereomicroscope (Zeiss) and photographed with a Hasselblad camera adapted for Bellows and Zeiss lummar lenses.

## RESULTS

#### Complications and mortality

Anesthetic complications constituted a major problem, especially initially, and it was found that a safer and deeper general anesthesia was obtained by diluting the Mebumal from 30 to 15 mg per ml and combining it with ether and narcosis. Animals dying from anesthetic complications prior to the completion of the surgical procedure were not recorded and are therefore not included in this material. Operative complications were very rare. Some animals where the humeral autografts exceeded more than half the circumference of the humerus developed a fracture of the humerus but after about 3 weeks could again use the limb. The blood loss during surgery was usually minimal but 3 animals developed large hematomas which had to be removed from the mandibular wound. In spite of the penicillin treatment, 6 animals developed an infection in the mandibular wound. Three of these animals developed so extensive an abscess formation that the healing process was seriously impaired and thus had to be excluded from the survey.

Out of 52 animals operated, 16 died during the observation period. Of these, 1 died some hours after the operation probably due to an anesthetic complication or a fat embolus, 2

died of toxicity of hematoporphyrin, a bone labelling agent used for fluorescence microscopy, 1 died for a reason unknown, and 12 died of an epidemic hemorrhagic enteritis.

All animals that expired before the end of the observation period and the 3 above mentioned heavily infected animals and one animal where the angiogram was not considered representative, were excluded from the investigation. The total number of animals used for analyses was 33, of which 19 underwent an implant from the humerus and 14 underwent an implant from the iliac crest (Table I).

The animals were unwilling to eat during the first postoperative days but could usually eat carrots or animal pellets on the third day. As a rule they lost 2-3 hg during the first week but usually regained weight subsequently.

#### *Microscopical observations*

A detailed report on the observations made by light and fluorescence microscopy will be given in a following publication. Only data relevant to the pattern of circulation are discussed in this paper.

### MICROANGIOGRAPHY

#### *Nine Days Postoperatively*

##### *Humeral inlay*

A network of fine capillaries penetrated the gap between the graft and the host bone perpendicularly from both the medial and lateral sides though more abundantly from the medial side. The vessels radiated along the endosteal side of the graft and sometimes completely filled the former medullary cavity of the grafted humerus (Fig 2). Revascularization of the disrupted apical pulp vessels had also started at that time. The cortex of the graft was still avascular except in one animal where tiny capillaries pierced the cortex from both the endosteal and periosteal sides. The periosteum of the graft was in all cases supplied by wide vessels, in particular on the medial side. In all cases there was a good correlation be-

Table I *Material of rabbits used for physiological studies*

Observation time (weeks)	Humeral inlay		Iliac crest
	Histology	Microangiography	Histology
1	5	5	3
2	3	4*	3
3	4	4	3
4	6*	5	5
Number of investigated rabbits	18	18	14
Total number of investigated rabbits	Humeral inlay 19		1

\* Only microangiography performed in  
\* Only histology performed in one rabbit  
roangiogram was not considered representative

tween the formation of woven bone vascularity (Fig 3), although of cartilage formation and subsequent chondral ossification on the periosteal

##### *Iliac inlay*

The ingrowth of capillaries was here considerably sparser but followed the term as described above. The vessels penetrated along the osteotomy line some cases grew down some fractions millimeter along the trabeculae of the (Fig 4). The vascular supply to of the graft was scanty and in some vessels had not yet reached the periosteum. The osteogenesis was retarded in all cases due to an inadequate blood supply (Fig 5) & there were no signs of cartilage formation.

Fig 2 Spalteholz preparation showing a 0.8 mm thick cross section of a humeral graft inserted into the mandibula after 1 week. Note the penetration of vessels from the medial side (M) into the medullary cavity (MC). There is a nascent revascularization of the pulp vessels (PV) while the humeral cortex (HC) is totally avascular. Magnification  $\times 12$ .

Fig 3 Histologic preparation from the same animal as in Fig 2 demonstrating the periosteal callus (PC) between the host bone invading the medullary cavity from the medial side (M). Endochondral ossification (EO) is seen on the periosteal side of the humeral graft. Nascent ossification of the humeral cortex (HC) is also seen. Magnification  $\times 12$ .

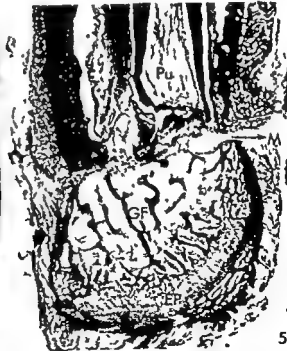
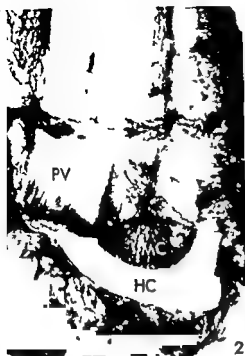


Fig. 2 Microangiogram showing the almost avascular iliac graft (IG) after 1 week. The vessels are penetrating the osteotomy line from the medial side (M) and start regularization of pulp vessels (PV). The vessels have not pierced the deeper layer of the periosteum (P) of the host fragment (HC). Spalteholz preparation 0.65 mm thick in cross section. Magnification  $\times 15$ .

Fig. 3 Cross section of histologic preparation of the same animal as in Fig. 4 with good periosteal callus (PC) on the medial side (M) of the host fragment. The osteotomy line is filled with woven bone on the lateral side but in this specimen there is only granular tissue on the medial side. The epiphyseal plate (EP) within the graft fragment is not closed. Magnification  $\times 15$ .



*Sixteen Days Postoperatively**Humeral inlay*

The vascularization around the cortex of the graft was by now intense and the vessels were wide and tortuous. They completely filled the former medullary cavity of the graft and had with slender capillaries pierced the graft cortex from both the endosteal and periosteal sides. These vessels did not form an anastomosing network within the graft cortex (Fig. 6). The lower third of the pulp was now occupied by wide capillaries. The periosteum was also completely vascularized, but there were still signs of endochondral ossification on the periosteal side. The development of trabecular bone surrounding the graft was abundant (Fig. 7) and the osteotomy line was hardly visible, though the bone formation within the graft cortex was not pronounced.

*Iliac inlay*

The vessels penetrated deeper into the graft. There was a preponderance of a perpendicular downgrowth of vessels from the well vascularized gap between the graft and the host bone. The vessels infrequently pierced the thin cortex from the dilated periosteal vessels of the graft. The vasculature surrounding the graft was substantially less than when a humeral inlay was used, though the interior of the graft was intersected with anastomosing capillaries of varying size occupying the proximal third of the graft. In one animal the whole transplant was vascularized except for the epiphyseal plate (Fig. 8). The bone formation around the graft was not so intense as when a humeral inlay was used, although there was new bone laid down upon the trabeculae within the graft cortex (Fig. 9). No signs of cartilage formation were seen.

*Twenty-three Days Postoperatively**Humeral inlay*

In 2 of 4 animals an extensive circulation developed in the graft cortex. The circulation in the other two graft cortices was poorer. In

some cases, there was a deficient filling of vessels in the former medullary cavity of the graft. This deficiency became even evident in the pulp and the sinusoidal spaces of the interdental spaces of the host bone (10). If this was due to intravital or post-thrombotic could not be determined by the graphic appearance. The histological showed a good endosteal bone formation, an evident resorption of the graft trabeculae within the graft cortex was pronounced even here. On the periosteal side there was no cartilage formation.

*Iliac inlay*

In one animal there was a poor vascularization to the graft. In the other two animals there was interlaced with a mesh of anastomosing capillaries occupying the upper two-thirds of the graft (Fig. 11). Even here there was reduction of the filling of the vessels of the pulp of the teeth. The vessels penetrated the graft in the same fashion as described previously, though in one animal the vessels traverse the cortex from the well vascularized periosteum. Only few vessels were seen to pass the epiphyseal plate. Though there was an abundance of vessels in the junction between the graft and the host bone, there was a mixture of dense fibrous tissue and trabecular bone. New bone was laid down upon the vascularized trabeculae inside the graft. Cartilage growth was observed.

---

Fig. 6 Microangiogram from a Spalteholz preparation of a 0.7 mm thick cross section showing abundant vascularization around the humeral graft after 2 weeks. The good revascularization of the pulp vessels (PF) is evident. Magnification  $\times 125$ . The inset above shows good vascularization of the intercortical capillaries within the graft from both the endosteal and periosteal sides. Magnification  $\times 17$ .

Fig. 7 Cross section of histologic preparation from the same specimen as in Fig. 6. The periosteal callus on the medial side (M) of the graft was accidentally removed when the osteosutures were taken out. The medullary cavity (MC) is completely filled with trabecular bone. Magnification  $\times 95$ .



Fig. 8 Microangiogram from a Spaltcholz preparation 8 mm thick in cross section demonstrating almost total evascularization of the iliac graft (IG) after 2 weeks. The bony cortex (C) on the lateral side (L) is not so well vascularized and there are no vessels traversing the epiphyseal plate (EP). Magnification  $\times 13$ .

Fig. 9 Cross section of histologic preparation from the same specimen as in Fig. 8. Note the broad periosteal callus (PC) from the medial side (M) of the host bone. No new bone formation is seen from the periosteum (P) of the graft. Magnification  $\times 9.5$ .

*Thirty Days Postoperatively**Humeral inlay*

In 4 animals of 5 investigated there was a marked increase in the vascularity of the graft. The vessels in the marrow of the grafted humerus were dilated and well filled. The periosteal circulation was intense and the circulation within the cortex of the graft appeared in some cases to be as vital as the circulation in the cortex of the host bone (Fig. 12). Quite often an extravasation of Indian ink into resorption cavities in the graft cortex was noticed. There was a good filling of the sinusoidal interdental network and the pulp cavity was in some cases supplied to its whole extent by capillaries, though by much fewer than normally. The histological picture showed an intense osteogenesis both endosteally and periosteally without cartilage formation. The graft cortex had undergone advanced resorption and subsequent bone formation.

*Iliac inlay*

In 2 of the 5 investigated animals, there was a complete filling of anastomosing vessels within the graft. However, few vessels had been able to penetrate the epiphyseal plate in these cases (Fig. 13). In one animal, the graft was supplied with wide, tortuous vessels in the upper half of the graft but only slender capillaries were observed further down. In two specimens the graft was poorly vascularized with slender capillaries.

The capillaries penetrated the graft, in the same manner as described previously, from the gap between the host bone and graft down and along the trabeculae of the graft, and very seldom did the vessels pierce the thin cortical layer from the periosteal side of the graft. The histology showed often a mixture of dense fibrous tissue and newly formed bone in the gap between the graft and the host bone. There was a good bone formation upon well nourished trabeculae within the graft. Cartilage growth with subsequent endochondral ossification occurred in one case only.

## DISCUSSION

*Comments on the microangiographic method*

There are various methods of visualizing microcirculation of bone tissue. Seven investigators have noted an insufficient visualization of the intracortical system when Micropaque (Gothman, 1961, Trueta & Danckwardt-Lilliestrom (1969) suggested this contrast medium during the first week following operation might have greater difficulty in passing the subperiosteal bone vessels than did Indian ink. Bränemark et al (1968) found lacking penetration of Indian ink, which was ascribed to intravascular aggregation of carbon. Another disadvantage with the Indian ink infusion method is the necessity for thick specimens. Perfusion with Microfil (coloured silicon rubber) maintains its properties during decalcification and sections  $\mu\text{m}$  or less can be made (Hunsuck, 1969). A pilot study performed by the author comparing the Microfil and Indian ink infusion techniques did not show any remarkable difference in visualization of the microvasculature in jaw bone. Rubin et al (1964) have stressed the importance of employing a physiological perfusion technique. Due consideration has been taken in this respect. Thus, the injection of Heparin 10000 IE reduced the occurrence of post-mortem thrombi. The infusions were always made in the direction of flow in the bloodstream, and with pressures never exceeding that of the systolic blood pressure. The duration of infusion was more than 1 hour, which led to a good filling of the vessels studied.

Fig. 10 Spalteholz preparation 1 mm thick in cross section showing good revascularization of the intramedullary vessels of a humeral graft after 3 weeks. The deficient filling of the medullary cavity (MC) by the vessels (PV) and the periodontium (Pe). Magnification  $\times 100$ .  
Fig. 11 Spalteholz preparation 0.6 mm thick in cross section demonstrating capillaries growing down the graft (UG) after 3 weeks. There is an avascular zone centrally and distally in the graft. Magnification  $\times 100$ .

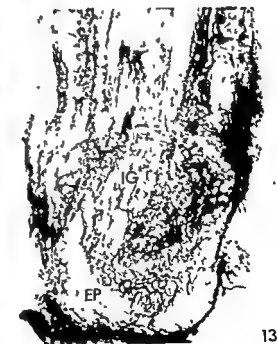
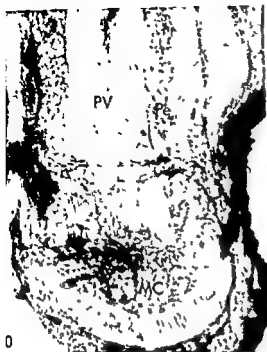


Fig 10 Spalteholz preparation 0.75 mm thick in cross section showing good revascularization of a humeral graft after 4 weeks. Note the rich revascularization of the pulp vessels (PV). Magnification  $\times 11$ . The inset above demonstrates thin capillaries piercing the graft cortex mostly from the endosteal side. The arrows along the endosteal border of the graft indicate extravasation of Indian ink into resorption cavities. Magnification  $\times 17.6$ .

Fig 13 Spalteholz preparation 0.7 mm thick in cross section showing good revascularization of a humeral graft after 4 weeks. The inset above demonstrates thin capillaries piercing the epiphyseal plate (EP). Magnification  $\times 9$ .

### *Pattern of vascularization*

The present investigation shows, in accordance with other investigations, that the invasion of vessels to the graft fragment occurs from the interfragmentary region and in particular from the medial aspect. This is probably due to an impaired circulation on the lateral side caused by the surgical trauma, while the circulation from the well nourished mucous membranes of the mouth remains. It is also shown by this study that during the observation period there was an increasing vascularization of both a longitudinally split full thickness autogenous humeral inlay and a full thickness autogenous inlay from the iliac crest. On the whole, the humeral graft became earlier and more completely surrounded by functioning vessels than the iliac graft. The reasons for this are probably various.

Firstly, the shape of the humeral inlay fits the defect created in the lower jaw better than does the iliac graft, which often is too narrow at its base. This causes often a poor approximation to the head fragment with mobility and a subsequent tipping of the graft.

Secondly, the vessels at once reach the humeral graft from all directions while the vessels from the medial side have a longer distance to go before reaching the narrow iliac graft, which often is placed in absolute contact with the host bone on the lateral side. The vessels also had a longer way before reaching the most distal parts of the interior of the iliac graft. It has been shown that if the marrow circulation becomes seriously impaired the original centrifugal bloodflow to the cortex to a major part will be replaced by a centripetal bloodflow from vessels in the surrounding soft tissues (Brookes et al., 1961, Rhinelanders, 1968, Dankwardt-Lilliestrom et al. 1970). In the present investigation, the main flow was in a centrifugal direction on the endosteal side of the graft but when a humeral graft was used there was in addition a considerable centripetal flow from the vessels in the enveloping soft tissues via the periosteum towards the cortex. In accordance with the accepted belief,

the interior of the iliac graft after four weeks was supplied by a richer network of capillaries than was the cortex of the humeral bone. Obviously, the reason for this is the more regular texture of the iliac graft where the vessels without any resistance can grow down all along the graft. The vessels have greater difficulties to anastomose in cortical bone because they must first bore out cavities through the bone to make their way.

Thirdly, the better revascularization of the periosteum of humeral grafts could possibly depend on a more abundant capillary supply in this kind of periosteum per se. This might be explained by the greater functional demands upon the humerus, which in the rabbit is the chief support of the foreleg, whereas there are no such demands upon the iliac crest. Meister (1914) showed the better regenerative powers of the humeral periosteum in comparison to ulnar periosteum in dogs. Rees et al. (1972) showed the superiority of the periosteum as compared to the periosteum of calvarian bone in rabbits as regards the osteogenic potency. Another barrier to the ingrowth of capillaries from the periosteum of an iliac graft consisted of the epiphyseal plate which rarely and then first after three to four weeks made room for vessels in either direction.

In the present study there were clear signs of resorption of the humeral graft by the widening of Haversian vessels already after 14 days, but hardly any vessels had at that time penetrated into the graft. This is, according to Brookes (1971), a characteristic reaction of compact bone to medullary ischemia which can proceed without signs of any remarkable osteoclasia. He suggests that the acid phospholipids and protein breakdown products caused by profound ischemia provide the initial stimulus for the formation of a reparative osteogenic blastema. This grows towards the ischemic area bringing with it abundant new vascular channels. In the present investigation there was an increased filling of the intracortical vessels from the second week onwards when humeral grafts were used. The

ar network showed the same architecture as the normal mandibula. These support the conception that the widening of Haversian vessels within the grafted facilitates the recanalization of the pre-existing Haversian system. Albrektsson (1971) stated that structures lining the vascular spaces of fresh bone grafts had ATPase activity at a time presumably preceding that of alveolar ingrowth, thus suggesting partial survival of the vascular endothelium of the graft. Early invasion of host tissue into the graft by means of recanalization of pre-existing graft vessels has been propounded by several investigators (Hancox, 1947; Stringa, 1965; Deleu & Trueta, 1965; Olerud & Dank-Lilliestrom, 1971).

#### *Osteogenesis and vascularity*

Osteogenesis and vascularity are closely related, which has been clearly shown by the work of Trueta & Cavadias (1955) and Latta (1963). Gothman (1961) found the live-arterial response related to regions where adequate callus was heaviest in tibial fractures in the rabbit and monkey. Maurer et al. (1965) showed that the formation of adequate callus always was preceded by a stage of hypervascularization. Zuckman et al. (1965) claimed that though the development of new formed vessels was a necessary pre-requisite stage for osteogenesis, it was not sufficient in itself. Thorogood & Craig (1975) studied through both light microscopy and electron microscopy that vascularization preceded the osteogenesis in bone graft. In the present study there was a strong correlation between the early revascularization of humeral graft and both bone resorption and new bone formation. The humeral bone graft early became surrounded by a dense capillary network and the new bone formation followed in its wake. As regard iliac grafts, new bone formation growing from the graft lined down and along the graft following the same paths as the capillaries could be observed. The vascularization of

the gap between the graft and host bone was in all cases more pronounced when a humeral graft was used. The development of trabecular bone was accordingly more decided in these cases, thus resulting in a better bony attachment of the graft to the host bone. The iliac grafts were, in fact, united to the host bone by a mixture of dense fibrous tissue and cancellous bone.

#### *Cartilage formation*

Cartilage formation often occurs in fracture repair and bone transplantation. The extent to which this occurs, or why it occurs at all, is still an unsolved problem. Leriche & Policard (1926), Unist & McLean (1941) claimed that cartilage develops in fractures as a result of inadequate immobilization. Ham (1930) and Girgis & Pritchard (1958) found cartilage formation in fracture repair when the blood supply was insufficient. Reddi & Huggins (1975) have described the transformation of fibroblasts to chondroblasts with subsequent endochondral ossification as the vascularity increases. In the present investigation, there were signs of cartilage formation with subsequent endochondral ossification in almost all humeral grafts during the two first weeks. This occurred exclusively on the periosteal side. This reaction subsided after two weeks and was not seen in any case after 3 weeks. Neither cartilage nor endochondral ossification was to be seen when an iliac graft was used, except in one case which occurred after 4 weeks. Thus the explanation for cartilage formation with endochondral bone formation could not be imperfect immobilization, because there were never any signs of displacement of the humeral graft. Even though the blood supply in this group seemed adequate as judged from the angiographic appearance, the callus formation on the periosteal side grew so rapidly that the blood vessels could not keep up with the osteogenic cells which under these circumstances became transformed into chondroblasts. This is in accordance with the theory proposed by Ham & Harris (1956).

more difficult to explain why there was no cartilage formation when iliac grafts were used. The retarded revascularization in combination with a paucity of osteogenic cells in this kind of periosteum might be one of the probable causes of this phenomenon.

There are many methods of improving the blood circulation to a free bone graft. Baadsgaard & Medgyesi (1965), Baadsgaard (1970), Medgyesi (1973) found improved and more rapid revascularization by using a bone graft with its vascular supply mediated through a muscle pedicle. Strauch, Bloomberg and Levin (1971) have explored the possibility of using a vascular island composite rib graft to the mandibula in dogs. They reported good survival of the bone graft and patency of the supplying blood vessels. Recently, Ostrup (1975) showed good survival of a composite rib graft in dogs by means of a microvascular anastomose between the intercostal and lingual vessels. Though the results from that investigation appear very encouraging, the method is laborious and takes a well trained and skilful surgeon to perform this operation. As shown from the present investigation, a bone graft implanted into well vascularized tissues will rapidly receive a blood supply penetrating into the bone marrow, while this happens more sluggish in the Haversian system of a compact bone. The choice of method for the clinical work will have to take into consideration the need for well vascularized tissue for bone transplantation. This is achieved in many flap techniques though the results vary (Millard et al. 1967; Millard et al. 1970; Conley, 1972; Conley et al. 1973). There might, however, be some cases where the surrounding tissues are sclerotic or else poorly vascularized where an intact circulation or microvascular anastomoses might give better results than free bone grafts.

## CONCLUSIONS

1. There was a progressive revascularization of both a split full bone autogenous humeral

graft and a full bone autogenous iliac graft when implanted into an artificial defect in rabbit mandibula.

2. The new capillaries arising from vessels in the soft tissues enveloping the jaw bypassed the graft during the first weeks mainly through the interfragmentary region and preponderantly from the medial side.

3. The periosteum of the humeral graft appeared earlier and more completely revascularized than when an iliac graft was used.

4. From the second week onwards periosteal vessels supplied the outer and cortical vessels of the humeral graft to an increasing extent, while they could hardly penetrate the epiphyseal plate of the iliac graft.

5. The penetration and downgrowth of capillaries were after 4 weeks more complete within the marrow of the iliac graft than within the humeral graft, which on the other hand came earlier and more completely surrounded by functioning vessels.

6. There was a good correlation between revascularization and osteogenesis within and around the grafts.

## ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Prof. Jan Wersall, the Karolinska sjukhuset, Stockholm, for guidance, encouragement and critical revision of the manuscript. I am also deeply indebted to Professor S. Olerud, Norrbacka Institutet, Stockholm, for revision of the manuscript. The skilful technical assistance of Sonja Björndahl and Bengt Hedberg is gratefully acknowledged. Ann Kempe has given me valuable help with the English.

## ZUSAMMENFASSUNG

Die Revascularisierung in zwei verschiedenen Arten Knochentransplantaten eingesetzt in einen künstlichen Schaden im Unterkiefer von Kaninchen wurde mit einer mikroangiographischen Methode untersucht. Eine Tusche in Arteria carotis infundiert wurde. Der Grad der Revascularisierung wurde zwischen einem autogenen Transplantat vom Humerus längs gespalten in 14 Fällen und einem autogenen Vollknochentransplantat aus der Crista ilica in 14 Fällen verglichen. Das Ausmaß des Gefäßwachstums wurde Woche für Woche bis Ende des ersten Monats nach der Operation kontrolliert. Man fand eine progressive Revascularisierung in beiden Arten

plantat. Die Periost des Humerustransplantates le früher und mehr vollständig als die Periost des atransplantates revascularisiert. Dagegen war die tration und das Herunterwachsen von Gefäßen voll iger in dem spongiösen Teil des Cristatransplantats. Einwachsen von Gefäßen wurde mit spezieller icht auf die Knochenneubildung in und rund der hiedenen Arten von Transplantaten diskutiert.

## REFERENCES

- e F H 1921 Certain fundamental laws underlying surgical use of the bone graft. *Ann Surg* 74: 196.
- Ekström M 1971 Repair of diaphyseal defects. Experimental studies on the role of bone grafts in reconstruction of circumferential defects in long bones. University of Göteborg.
- sgaard N 1970 Transplantation of pedicle bone grafts to fresh skeletal defects and defect pseudarthroses. *Acta Orthop Scand* 41: 261.
- sgaard N & Medgyesi S 1965 Muscle-pedicle bone grafts. An experimental study. *Acta Orthop Scand* 35: 279.
- kes M 1971 *The Blood Supply of Bone. An approach to Bone Biology*. Butterworths, London.
- kes M, Elkin A C, Harrison R G & Heald B 1961 A new concept of capillary circulation in one cortex. Some clinical applications. *Lancet* 1: 978.
- emark P I, Ekholm R & Lindhe J 1968 Colloidal carbon used for identification of vascular permeability. *Med Exp* 18: 139.
- ey J 1972 Use of composite flaps containing bone or major repairs in the head and neck. *Plast Reconstr Surg* 49: 522.
- ey J, Cinelli P B, Johnson P M & Koss M 1973 Investigation of bone changes in composite flaps after transfer to the head and neck region. *Plast Reconstr Surg* 51: 658.
- ward L, Lilliestrom G 1969 Reaming of the medullary cavity and its effect on diaphyseal bone. A fluorochromic microangiographic and histologic study on the rabbit tibia and dog femur. *Acta Orthop Scand* suppl 128.
- ward L, Lilliestrom G, Lorenz D L & Olerud S 1970 Intramedullary nailing after reaming. *Acta Orthop Scand* suppl 134.
- u J & Trueta J 1965 Vascularization of bone grafts in the anterior chamber of the eye. *J Bone Jt Surg* 7B: 319.
- s G G & Pritchard J J 1958 Experimental pro-
- Hancox M 1947 The survival of transplanted embryo bone grafted to chorioallantoic membrane and subsequent osteogenesis. *J Physiol* 106: 279.
- Hunsuck E E 1968 A method of quantitatively analyzing the microcirculatory architecture of the mandible. Preliminary report. *J Oral Surg* 26: 449.
- Lénche E & Policard A 1926 *Les Problèmes de la Physiologie Normale et Pathologique de l'Os*. Masson, Paris.
- Maurer P, Evrard J, Van Houtte & Mazabraud A 1963 Contribution à l'étude de la consolidation osseuse. Premiers résultats. Étude des réactions vasculaires au niveau et autour du foyer de fracture. *Rev Chir Orthop* 49: 689.
- Maurer P, Zucman J & Lewalle J 1965 Rôle de la vascularization périfRACTURE et centro-médullaire dans l'ostéogénèse réparatrice. *Rev Chir Orthop* 51: 229.
- Medgyesi S 1973 Observations on pedicle bone grafts in goats. Vascular connections between soft tissues and bones. *Scand J Plast Reconstr Surg* 71: 110.
- Millard D R, Dembrow V, Shocket E & Zaveritnik J 1967 Immediate reconstruction of the resected mandibular arch. *Am J Surg* 114: 605.
- Millard D, Garst W P, Campbell R C & Stokley S H 1970 Composite lower jaw reconstruction. *Plast Reconstr Surg* 46: 22.
- Olerud S & Danckwardt Lilliestrom G 1971 Fracture healing in compression osteosynthesis. An experimental study in dogs with an avascular diaphyseal intermediate fragment. *Acta Orthop Scand* suppl 137.
- Pfemister D B 1914 The fate of transplanted bone and regenerative power of its various constituents. *Surg Gynec Obstet* 19: 303.
- Reddi A H & Huggins Ch H 1975 Formation of bone marrow in fibroblast transformation ossicles. *Proc Nat Acad Sci USA* 72: 2212.
- Rhineland F W 1968 The normal microcirculation of diaphyseal cortex and its response to fracture. *J Bone Jt Surg* 50-A: 784.
- Ritsila V, Alhopuro S & Rintala A 1972 Bone formation with free periosteum. An experimental study. *Scand J Plast Surg* 6: 51.
- Rubin P, Casarett W, Kurohara S & Fujii M 1964 Microangiography as a technique. Radiation effect versus artefact. *Am J Roentgen* 92: 378.
- Spalteholz K W 1914 *Über das Durchsichtigmachen von Menschlichen und Tierischen Präparaten*. Zweite Auflage. S. Hirze, Leipzig.
- Strauch B, Bloomberg A E & Lewin M L 1971 An experimental approach to mandibular replacement. Island vascular composite rib grafts. *Brit J Plast Surg* 24: 334.
- Stringa G 1957 Studies of the vascularization of bone grafts. *J Bone Jt Surg* 39-B: 395.
- Thorogood P V & Gray J C 1975 The cellular changes during osteogenesis in bone and bone marrow composite autografts. *J Anat* 120: 127.
- Trueta J J 1963 The role of the vessels in osteogenesis. *J Bone Jt Surg* 45-B: 402.
- Trueta J & Cavadias A X 1955 V



- caused by the Küntscher type of nailing *J Bone Jt Surg* 37 B 492
- Unst M R & McLean F C 1941 Calcification and ossification. I. Calcification in the callus healing fractures in normal rats *J Bone Jt Surg* 23 A 1
- Zuckman J, Maurer P & Berbeson C 1968 The effect of autografts of bone and periosteum in recent diaphyseal fractures. An experimental study in the rabbit *J Bone Jt Surg* 50-B 409
- Östrup L T 1975 The free living bone graft. A experimental study. Linköping University Medical dissertations
- A Nathanson M D  
Dept of Otolaryngology  
Karolinska Sjukhuset  
S-10401 Stockholm  
Sweden

## CYTOLYTIC ACTIVITY OF TONSIL CELLS

E. V. Gjulling and O. F. Melnikov

*From the Department of Pathophysiology, the Institute of Otolaryngology, Kiev, USSR*

*(Received January 5, 1977)*

**Abstract.** The ability of normal canine tonsil cells and of humans with chronic tonsillitis to lyse heteroerythrocytes *in vitro* is shown. Heterologous erythrocyte lysis by tonsil cells is markedly increased after immunization of the animal. Tonsil cells of patients with chronic tonsillitis lyse the erythrocytes coated with streptococcus and staphylococcus antigens more actively. Experimental tonsillitis increases the ability of tonsil cells to lyse erythrocytes. Since cytolytic activity of tonsil cells is not altered by the removal of phagocytes and treatment with antiglobulin serum, one can presume that it is mediated by T lymphocytes.

Oettgren et al. (1966) showed that the palatine tonsils of patients with chronic tonsillitis contain 40% of lymphocytes, which can be interpreted as thymus derived cells because of their ability to transform into blasts being induced by phytohemagglutinin. This fact was further confirmed by the determination of lymphocytes able to form rosettes with sheep blood cells (Brown & Greaves, 1974; Gjulling, 1975) and with the help of specific antiserum (Greaves & Brown, 1974). The presence of the cells' thymic origin in animals is shown in experiments on thymectomized rabbits (Zuker & Iho, 1973) and dogs (Buchem & Gjulling, 1973). It has been established that the fact that T lymphocytes are lymphoid system cells prompts the suggestion that tonsils play an active part in cellular immunity reactions (Gjulling, 1972). Nowadays the ability of human and animal tonsil lymphocytes to cause various host reactions is accepted (Harrison,

1972; Nikolsky et al., 1975). It has been shown that a considerable proportion (up to one-third) of cells react by transformation to blast cells with streptococcus and staphylococcus antigens, in populations of small lymphocytes in tonsils of patients with chronic tonsillitis (Vizirenko & Gorshevikova, 1972; Gnnevitch & Vizirenko, 1973). It has also been demonstrated that the migration inhibitory factor is released from tonsil lymphocytes of patients with chronic tonsillitis under the influence of microbial antigens (Rocklin et al., 1972; Melnikov, 1973).

We have studied the cytolytic activity of tonsil lymphocytes that is, the most important index of cellular immunity tension. The results of the determination of the cells' capacity for non specific and immune cytolysis in the normal and inflamed state are given in the present report.

### MATERIALS AND METHODS

In 22 mongrel dogs (1-4 years old, weight 5-15 kg) cytolytic activity of tonsil cells was studied in intact dogs and animals immunized with  $10^7$  hen erythrocytes, injected into tonsils.

0.2 ml of mixture consisting of equal parts of complete Freund's adjuvant (Calbiochem) and a 24 hour culture of  $\beta$  haemolytic streptococcus and containing about 2 billion microbial bodies per 0.1 ml was injected into tonsils of 3

dogs to produce tonsillitis within one hour after injection of the heteroerythrocytes.

Six dogs of another group received the infective mixture twice. The second injection was given 2-3 weeks after the first. These dogs were immunized within a month of the second injection. Tonsils of experimental dogs were extracted 10-11 days after immunization.

Cytolytic activity of tonsil cells from patients with chronic tonsillitis was evaluated according to the results of the non-specific destruction of hen erythrocytes and to the index of cytolysis of erythrocytes coated with staphylococcus and streptococcus antigens. In the latter case, cytolysis may be a specific characteristic, as the microbes mentioned are believed to induce tonsillitis.

Cell suspensions of tonsils from 49 patients, 15-50 years of age and suffering from chronic tonsillitis for 5-8 years, were used.

Excised tonsils were washed in Hanks' solution with antibiotics (penicillin and streptomycin, 100 U/ml), cut very finely, and passed through a nylon filter into siliconized tubes.

The cell suspension was washed twice with Hanks' solution at 4°C by centrifugation (150 g, 3 min) and finally suspended to a concentration of  $5 \times 10^7$  cells/ml in the following: 87 ml of medium 199, 10 ml of fetal serum inactivated by heating, 100 fold concentration of L-glutamine, amino acids and vitamins per 1 ml, penicillin 100 U/ml, streptomycin 60  $\gamma$ /ml.

A portion of tonsil cells was treated with carbonyl ferrum in order to remove phagocytes from the suspension in a magnetic field by the method of Kupers et al (1961). Another phagocyte-depleted portion of tonsil cells was incubated with complement and human globulin antiserum at 37°C for an hour in order to block B cells. After that the cells were washed in tissue culture medium twice for 3 minutes at 150 g.

Erythrocytes of chicks not more than 4 months old were used as target cells. Erythrocytes obtained in sterile conditions were conserved in 0.85% sodium chloride solution with

**Table 1** Cytolytic activity of tonsil cells from intact dogs and from dogs immunized with chicken erythrocytes

Statistical indexes	% Isotope ( $^{51}\text{Cr}$ ) release from target cells			
	Groups I	II	III	IV
<i>M</i>	3.5	11.1	25.0	24.6
<i>+m</i>	1.9	1.6	3.8	4.2
<i>n</i>	8	6	3	5
<i>P</i>	-	-	<0.05	<0.05

I: intact animals, II: normal immunized dogs, III: dogs immunized before induction of tonsillitis, IV: dogs immunized after second induction of tonsillitis.

25 U/ml heparin for up to 48 hours. Treated hen erythrocytes were coated with complete streptococcus and staphylococcus antigens obtained by the method of Vershup (1969). These erythrocytes were agglutinated with homologous serum (titres 1:16-1:128) in a passive haemagglutination reaction. Erythrocytolysis induced by lymphocytes was determined according to the recommendations of Perlmann et al (1970).

The basic experiment was conducted in neutral glass bottles by adding chicken erythrocytes ( $10^6$ ) to a lymphoid cell suspension (1) in the ratio 10:1 and a constant volume of medium of 1 ml.

Killed lymphocytes, or  $2 \times 10^7$  non isotope labelled chicken erythrocytes, were added to control bottles. The bottles were incubated 24 hours at 37°C and the percentage of isotope release as regards the spontaneous release in controls was determined according to the method of Perlmann et al (1970).

The results were evaluated by Student's test.

## RESULTS

The data presented here indicate the ability of normal dog tonsils and those of people with chronic tonsillitis to lyse heteroerythrocytes *in vitro* (Tables I, II). After immunization

mals with heterologous erythrocytes, destruction of the latter by tonsil cells increases markedly (Tables I, II). Tonsil cells of patients with chronic tonsillitis lyse more actively the erythrocytes coated with streptococcus and staphylococcus antigens (Table I).

An experimental tonsillitis increases tonsils' capacity for erythrocytolysis (Table I).

The removal and treatment of phagocytes with antoglobulin serum does not adequately affect the cytolysis of heteroerythrocytes by tonsil cells.

## DISCUSSION

The non specific destruction of various target cells by lymphocytes has already been noted and recognized as a manifestation of the phenomenon of allogenic inhibition (Utsui & Everett, 1974; Hayward, 1974) or 'non relevant' killing (Willumsen & Heron, 1974). Erythrocytolysis brought about by tonsil cells is probably similar to or identical with the phenomenon described. By its nature, an adequate increase in the cytolytic activity of tonsil lymphocytes caused by preliminary immunization of dogs by target cells, is evidence of the tonsils' capacity for cellular immunity reactions.

An increased destruction of heteroerythrocytes coated with microbial antigens by tonsil lymphocytes in patients with chronic tonsillitis cannot be explained as being a result of their exposure to the above mentioned microbial antigens, as no increase in spontaneous lysis of the treated erythrocytes is noted in the controls. The cytolytic effect is not associated with phagocytosis of target cells either since phagocyte removal does not affect the level of erythrocytolysis. As patients with chronic tonsillitis almost always have antibodies to streptococcus and staphylococcus one can only consider the destruction of erythrocytes coated with antigens to these microbes to be not only the result of immune cytolysis caused by the sensitized lymphocytes but also the

Table II Cytolytic activity of tonsil cells in presence of chronic tonsillitis

Statistical indexes	% Isotope ( $^{51}\text{Cr}$ ) release from target cells				
	I	II	III	IV	V
$\bar{M}$	10.5	25.4	23.6	27.2	35.6
$\pm m$	1.4	3.6	4.1	2.6	7.0
$n$	39	37	38	10	5
$P$	-	<0.05	<0.05	<0.05	<0.05

I - chicken erythrocytes + tonsil cells; II - chicken erythrocytes coated with staphylococcus antigens; III - chicken erythrocytes coated with streptococcus antigens + tonsil cells; IV - chicken erythrocytes coated with streptococcus antigens + tonsil cells without phagocytes; V - chicken erythrocytes coated with streptococcus antigens + treated with antoglobulin serum tonsil cells without phagocytes.

product of cytolytic activity of cells with membrane receptors to Fc fragments of IgG.

Treatment of tonsil cell suspension with antoglobulin serum capable of blocking II cells (Resch et al. 1974) does not suppress the erythrocytolysis. This fact permits us to consider the T lymphocyte to be mainly an effector cell.

The increase in erythrocytolysis in the presence of chronic tonsillitis may be caused by an accumulation of lymphoid cells, presumably T population in the focus of inflammation (Fedotov 1969; Asherson & Alwood 1973).

## ZUSAMMENFASSUNG

Die Zellen der Gaumenmandel von gesunden Hunden und der Leute mit Tonsillitis können die heterologischen Erythrozyten ( $^{51}\text{Cr}$ ) *in vitro* zerstören. Nach der Immunisierung der Tiere war die cytolytische Aktivität viel größer. Die Bearbeitung der Erythrozyten von Hunden mit den antigenen Streptococcus haemolyticus oder Staphylococcus aureus hat Bedeutung und zur Zerstörung solcher Erythrozyten geführt. Die experimentelle Entzündung in den Tonsillen hat die cytolytische Aktivität erhöht. Die Entfernung der Phagozyten im magnetischen Feld und auch die Bearbeitung der Lymphozyten aus Tonsillen von dem Serum gegen menschliche Globuline hatten die cytolytische Aktivität nicht verändert. Nach der Meinung der Autoren wird diese Aktivität durch T Lymphozyten hervorgerufen.

## REFERENCES

- Asherson, G & Alwood G 1973 Movement to sites of inflammation and non specific cytotoxicity of T-lymphocytes stimulated by antigen in vivo and their resemblance to lymphocytes stimulated with PHA in vitro. *Int Arch Allergy* 41: 265-272.
- Buchner, F & Kuipers W 1973 On the origin of lymphocytes cells in the palatine tonsil *Acta Otolaryngol* (Stockh) 73, 527.
- Grinevitch Yu A 1975 *Zh Ushn Nosov Gorlov Bolez* 4, 48.
- Harrison B 1972 Antibody production in thymectomized mice with implants of rabbit tonsillar tissue *Transplants* 14, 402.
- Hayward A R 1974 Non specific induction of cytotoxicity by human lymphocytes with human IgG *Immunology* 26, 61.
- Kupers, S, Bignall, I & Luckock, D 1961 A quantitative method for studying tumor cells in blood *Lancet* 83, 852.
- Melnikov, O F 1973 *Zh Ushn Nosov Gorlov Bolez* 4, 116.
- Nikolsky, I S, Novokhatsky, A M, Shekman, A M & Grudzinsky, G S 1975 *Zh Ushn Nosov Gorlov Bolez* 5, 55.
- Oettingen, H, Silber, H, Miescher, P & Hirschorn R 1966 Stimulation of human tonsillar lymphocytes in vitro *Clin Exp Immunol* 1, 77.
- Perlmann P, Perlmann H, Wasserman I & Palen, T 1970 Lysis of chicken erythrocytes sensitized with PPD by lymphoid cells from guinea pigs immunized with tubercle bacilli *Int Arch Allergy* 204.
- Resch, K, Gelfand E & Prester, M 1974 Antibody mediated target cell lysis by non immune cells in the presence of anti immunoglobulin to distinguish effector cell population *J Immunol* 112, 792.
- Rocklin E, Remold H & David J 1972 Characterization of human migration inhibitory factor (MIF): antigen stimulated lymphocytes *Cell Immunol* 436.
- Suzuki, M & Iho H 1973 Relation between thymus tonsil *Arch Klin Exp Ohren Nasen Kehlkopf* 204, 249.
- Tsutsui, I & Everett N 1974 Specific versus non specific target cell destruction by T lymphocytes sensitized in vitro II Responsible factors for non specific destruction *Cell Immunol* 10, 359.
- Vershigora A E 1969 Doctoral dissertation Kiev.
- Vizurenko, L V & Gorshevikova E V 1972 *Zh Ushn Nosov Gorlov Bolez* 2, 52.
- Willumsen, I & Heron I 1974 Cell mediated lysis in man: A case of non relevant killing of party persons *Tissue Antigens* 4, 172.

E V Gulling MD  
Dept of Pathophysiology  
Institute of Otolaryngology  
Kiev  
USSR

# PERIPHERAL VASOCONSTRICTION IN THE RAT IN RESPONSE TO SOUND

## I *Dependence on Stimulus Duration*

E Borg

*From Department of Physiology II, Karolinska Institutet, Stockholm, Sweden*

(Received June 18 1977)

Vasoconstriction in response to sound has been in the non-anaesthetized rat. Arterial pulsations were recorded by a non-invasive technique: band noise bursts at 80 dB SPL, with durations 1 ms to several hours were presented in a free sound isolated box. The results show that energy is integrated with a time constant of about 1 s. The responses during continuous stimulation had slowly and the time to halfway normalization of pulsation was more than 15 min.

found capable of eliciting vasoconstrictions at sound levels down to the threshold of hearing. The degree of vascular reaction, measured as the duration of the response, increased approximately linearly as a function of sound level, at least over 80 dB (sound pressure level, re 20  $\mu$ Pa). The latency time was about one second, and the total duration of the response exceeded one minute. With presentation intervals of 10-15 minutes there was insignificant habituation over an 8-10 h period for 80 dB SPL noise bursts. This acoustic-vascular reflex was suggested as a suitable model for studies of influence of sound on autonomic functions.

The aim of the present study is to determine how the vascular response varies as a function of the duration of a broad band noise signal of 80 dB SPL.

Two experiments were performed: (a) temporal integration was investigated for short noise bursts, 1-4000 ms, (b) adaptation, or habituation of the vasoconstriction was determined during prolonged sound exposure.

## MATERIAL AND METHODS

The experiments were performed on 13 adult male Sprague-Dawley rats. Five of them were

This study was supported by the Swedish Work Environment Fund, project No. 74/24.

tail of the rat has a rich vascular supply, serves an important function in thermoregulation (Rand et al., 1965). The blood flow may vary more than one hundredfold in response to different demands on heat dissipation (Johansen, 1962). When room temperature is normal, the tail arteries are constricted, the flow is low. Above an ambient temperature of about 30°C the artery dilates and heat is lost. When dilated, sensory stimuli of various types, especially a drop in temperature but also sound and painful stimuli, elicit constriction of the vessels in the tail. Some basic characteristics of the acoustically elicited vasoconstriction of the rat's tail vessels have recently been described, together with a technique allowing repeated recordings of this reflex in the non-anaesthetized rat (Borg, 1977). The blood flow in the tail artery was indirectly assessed in terms of amplitude of pulsations recorded from the surface of the tail. A short burst of broad-band noise was



Fig. 1 Recordings of arterial pulse from the tail of a non-anesthetized rat. 80 dB SPL noise burst elicits a vasoconstriction quantified by  $T_{1/2}$  time from stimulus onset until pulse amplitude is 1/2 normalized. Stimulus duration (10 or 4000 ms) is indicated below each trace.

experimentally naive, the remainder had been subjected to similar experiments earlier. The measurements were separated in two sessions, 10 of the animals participating in both of them.

The technique has been described in detail earlier (Borg, 1977) and only some essentials will be outlined here. During the experimental session the animal rested in an individually adjustable net tube on a regulated heating pad. The blood flow in the tail was assessed in terms of pulsations recorded by a rubber balloon connected to a volume-sensitive transducer (Elema 510C). Sound stimuli were presented through a Lansing L 75 loudspeaker 10 cm in front of the rat and consisted of 80 dB

SPL broad band noise bursts of varying duration. The maximum energy was in the range 3–15 kHz (see figure 2 in Borg, 1977) and rise and decay times of the bursts were  $\leq 1$  ms. The bursts were presented with approximately 15 min intervals and each experimental session lasted between 8 and 12 hours.

The duration of the noise burst was 1, 20, 50, 100, 1000, or 4000 ms in the first experimental session. Each burst was presented several times in random order. In the second experiment, some weeks later, bursts of four duration, namely 1, 10, 100 and 990 ms were presented at random and finally a continuous noise was introduced. In this latter session

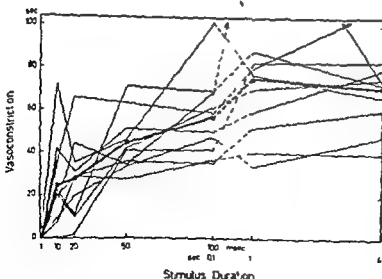


Fig. 2 Duration of vasoconstriction ( $T_{1/2}$ ) as a function of duration of 80 dB SPL noise bursts in 10 rats. Each line represents one experiment. Dashed line shows average. Abscissa is drawn with two scales. Two values of  $T_{1/2}$  are shown in each frame.

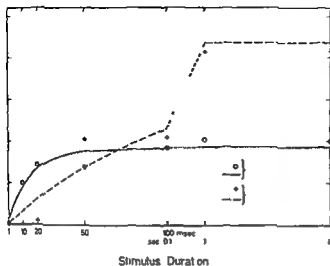


Fig 3 Observed durations of vasoconstriction in response to 80 dB SPL noise bursts of different duration for 2 animals (+ and o) and the adapted exponential function  $y = a[1 - \exp(-(1/T)x)]$  where  $a$  is the asymptote and  $T$  is time constant. The fitness was 60% and 87% and the estimated  $T$  was 125 and 16 ms respectively.

or a few presentations were made at setting. The vasoconstriction was quantified as the duration of the response from the onset of the sound until the amplitude of the response had returned halfway to the pre-stimulus level ( $T_{1/2}$  in Fig 1).

Stimuli were presented only when certain criteria were fulfilled: the pulse recording had to be free from artifacts indicating ongoing vasoconstriction or activity of the rat; responses were discarded if the initial vasoconstriction was obscured by subsequent spontaneous responses before the pulse amplitude had returned halfway to pre-stimulus level (see further Borg 1977). Less than 10% of the responses had to be discarded for that reason in the first experiment, and none in the second experiment.

## RESULTS

### Response to short noise bursts

Recordings of arterial pulsations obtained from the surface of the tail of the rat are shown in Fig 1 which illustrates typical effects of 80 dB SPL noise bursts of different durations (10, 100, 4000 ms). About 1 s after the onset of the noise burst (indicated below each recording) the pulse amplitude declined. After about 1 min it had recovered completely. As a

rule, a 10 ms noise burst elicited a smaller and shorter reaction than bursts in the range of 100–4000 ms.

The duration of vasoconstrictions in response to stimuli of different lengths is shown in Fig 2 as thin continuous lines for the 10 individual rats. The heavier line shows estimated mean values. It is seen that the individual variability is considerable, but generally the short sounds elicited smaller responses than sounds of 50–100 ms duration. The average curve more clearly illustrates the temporal integration in the vasoconstriction reflex.

In order to obtain an estimate, at least a

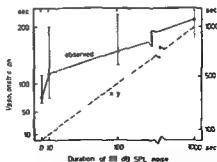


Fig 4 Duration of vasoconstriction in response to extended 80 dB SPL noise bursts: median and semi-interquartile range ( $\pm 25\%$ ). Each point represents 12 rats. Broken line shows hypothetical situation without adaptation: response being equal to stimulus. Left ordinate corresponds to stimulus duration 1, 10 and 100 s and right ordinate to 990 s bursts.



rough one, of the time constant for temporal integration in this reflex, each individual set of values was described by an exponential function,  $R=a[1-\exp(-t/T)]$ , where  $R$  is response duration,  $a$  corresponds to the horizontal asymptote,  $T$  is the time constant, and  $t$  is duration of stimulus. The parameters were determined by a non linear (Gaussian) process. In 5 of the animals the fitness was better than 60% and the time constant varied between 16 and 125 ms, averaging 60 ms (median 50 ms). The time constant of the system is then approximately 0.1 s.

Fig. 3 illustrates the adapted functions and the observed values for 2 animals. The fitness was in these cases 60% and 87% respectively, and the time constants were 125 and 16 ms.

### B Response to extended noise

The duration of the vasoconstriction elicited by a long stimulus, from the beginning of the sound to the moment the pulse amplitude first exceeded  $(A+B)/2$ , is illustrated in Fig. 4. During prolonged stimulation (100–990 s) the pulse amplitude often waxed and waned, exhibiting a sequence of vasoconstrictions. Such multiphasic responses have been excluded in the analysis of short bursts (Figs. 1 and 2, and Berg 1977). For the sake of consistency those and 10 s bursts followed by multiple constrictions were not excluded in Fig. 4 and the values for 1 (and for 10 s) are not directly comparable with the values for 1 and 4 s of Fig. 2.

The median and semi interquartile range from 12 experiments in 12 rats are illustrated. The broken line shows the duration expected to occur, should the system be completely without adaptation, i.e. duration of stimulus and response are equal. Responses to stimuli up to 100 s did not adapt. Several individual values were on the other hand considerably larger than expected and so was the median value. During 990 s stimulation the response habituated in 5 cases, after 122 to 207 s, but mostly it outlasted the noise considerably.

On continuous stimulation the pulse amplitude returned halfway to pre-stimulus value af-

ter, on the average, 16 min (median 11 min). Stable vasodilation was, however, not reached until after 47 min (median range 25–60 min). It is also to be noted that the control noise was presented after several stimuli, range 1 to 990 s. Rate of habituation therefore be overestimated in these experiments compared with habituation to a continuous "novel" stimulus. At any rate it is evident that even with a fairly short period of rest between presentations, responses only slowly habituate.

## DISCUSSION

The present results show that 1) noise with durations less than approximately 100 s are relatively inefficient for the production of vasoconstriction, 2) the vasoconstriction habituates, but slowly, when the rat is exposed to continuous 80 dB noise.

The variability in the responses is considerable (Figs. 2 and 3) and this technique is to be compared with many psychophysical methods with respect to reliability of measured value. In an earlier study (Berg 1977) the influence on the responses of various factors was analysed. The effect of variation in temperature and body movements increase variability without introducing systematic errors, since stimuli are presented in random order.

It is interesting, though, to observe that the time constant for integration of acoustic energy in this acoustic-vascular reflex is of the same order of magnitude as time constant for loudness (e.g. Zwischlocki, 1969). It remains to be settled whether the similarity of time constants signifies a functional relationship between acoustic perception and sound-elicited autonomic activation. A possible morphological substrate for such an interaction in the brain is already well established: the habundant innervation of the modulus in the inner ear (Spilner & Lichtensteiger, 1966; Densen 1971).

In the present experiments several prolonged noise presentations were made

short intervals (about 15 min), yet these only poorly adapting responses. It has been shown (Borg, 1977) that this does not habituate to 1 s bursts of 80 dB noise presented with 10–15 min interval at least 8–12 h. A peripheral vasoconstriction in response to sound has long been known in humans (e.g. Mosso, 1874, Jan- sen 1974) and is stable during prolonged exposure (Lehmann & Tamm, 1956). Although the reversibility of the vasoconstriction may be slow, sound has a permanent influence on the cardiovascular system, it is premature to state about harmful effects of environmental sound other than hearing loss.

### ZUSAMMENFASSUNG

An 12 chloralhydratisierten Ratten wurde die Gefäßkontraktion als Antwort auf Tongeräusche untersucht und zwar 1. Aufzeichnung der arteriellen Pulsationen im 2. mittels einer nichtinvasiven Methode. Als Stimuli dienten hochfrequente breitbandige Geräuschstöße in Paketen von einer Stärke von 80 dB SPL und unterschiedlichen Dauern von 1 ms bis zu mehreren Sekunden im freien Schallfeld in einer Schallbox. Das Resultat war, daß die akustische Energie integriert wird mit einer Zeitkonstante von etwa 0.1 s. Die Antwort auf kontinuierliche Stimulation habituierte nur langsam und die Kontraktion bis zum Amplitudenanstieg der arteriellen Pulsationen halbwegs auf den Ausgangswert betrug mehr als 5 min.

### REFERENCES

- Borg E 1977 Tail artery response to sound in the unanaesthetized rat *Acta Physiol Scand* 100 129  
 Densert O 1974 Adrenergic innervation in the rabbit cochlea *Acta Otolaryngol* (Stockh) 78 345  
 Jansen G 1974 Untersuchungen über die psychophysiologische Wirkung von Geräuschen mit unterschiedlichem Bedeutungsgehalt *Sozial und Präventivmedizin* 19 161  
 Johansen K 1962 Heat exchange through the muskrat tail. Evidence for vasodilator nerves to the skin *Acta Physiol Scand* 55 160  
 Lehmann G & Tamm J 1956 Über Veränderungen der Kreislaufdynamik des ruhenden Menschen unter Einwirkung von Geräuschen *Internat Z angew Physiol einschl Arbeitsphysiol* 16 217  
 Mosso A 1874 Von einigen neuen Eigenschaften der Gefäßwand *Berichte Math Phys Classe Königl Sächs Gesellschaft Wissensch* 26 305  
 Rand R P, Burton A C & Ing T 1965 The tail of the rat in temperature regulation and acclimatization *Can J Physiol Pharmacol* 43 257  
 Spoendlin H & Lichtensteiger W 1966 The adrenergic innervation of the labyrinth *Acta Otolaryngol* (Stockh) 61 423  
 Zwislocki J J 1969 Temporal summation of loudness. An analysis *J Acoust Soc Am* 46 431

E Borg MD  
 Dept of Physiology II  
 Karolinska Institutet  
 S 10401 Stockholm  
 Sweden

## TRANSTYMPANIC ELECTROCOCHLEOGRAPHY DURING GLYCEROL DEHYDRATION

D A Moffat, W P R Gibson, R T Ramsden,  
A W Morrison and J B Booth

*From the Department of Otolaryngology The London Hospital  
Whitechapel London Great Britain*

(Received January 20 1977)

**Abstract** Thirteen patients with Meniere's disorder were tested using transtympanic electrocochleography during glycerol dehydration. The most common finding was a decrease in the negative summing potential and this appeared to be a more sensitive indicator of changes occurring in the cochlea than pure tone audiometry and speech discrimination. A pathophysiological explanation for this observation is offered and the possibility of using this decrease in the negative summing potential as a pointer to the prognosis of endolymphatic sac surgery is discussed.

The diagnosis and treatment of Meniere's disease has both fascinated and frustrated physicians for many years. Diagnosis may be difficult because many conditions including syphilis, vertebro-basilar insufficiency, cochlear otosclerosis, perilymph fistula and acoustic neuroma may be characterised by Meniere's syndrome. Meniere's disorder is an idiopathic condition and can only be diagnosed after all the known causes of the syndrome have been properly excluded. Patients with an incomplete symptom complex are often diagnosed as Meniere's disorder in the belief that they represent early or even atypical forms of the disease but a proportion of these patients are inevitably incorrectly diagnosed. The assessment is also complicated by certain well known non-organic factors which have an association with the disorder namely personality type and the response of the patient

to environmental stress. A useful technique which appears to confirm the diagnosis in many cases is transtympanic electrocochleography (Gibson et al, 1976).

### PATHOGENESIS

Apart from the diagnostic problems there are considerable difficulties in the management of these patients. The long list of medical and surgical treatments for Meniere's disorder reflects our inadequate knowledge of the pathogenesis. Hallpike & Cairns (1938) first demonstrated the presence of endolymphatic drops in post mortem studies and this has been largely confirmed by subsequent workers. Many have attempted to correlate pathological changes with ante mortem symptoms (Knecht, 1963). The aetiology of the hydrops is not known but many attractive theories have been proposed which are either concerned with the production, reabsorption and excretion of the endolymph or with an alteration in the endolymph potential secondary to changes in ATP activated ion balance.

Spasm of the stria vascularis has attracted a great deal of attention. It may interfere with endolymph circulation, thus producing hydrops. Disorders of the endolymphatic duct have also been incriminated. The

is endolymphaticus may be an important  
endolymph reabsorption or may act as  
compensating for changes in endo-  
pressure. Malfunction may then lead  
olymphatic hydrops.

ly, doubt has been cast on the theory  
ymph actually circulates and it may  
the stria vascularis acts as an ATP  
lled electrotonic mechanism, which  
ns the ionic composition and resting  
al potential of the endolymph. It is pos-  
that no circulation occurs except in  
conditions when the saccus endo-  
may act as an important site of re-  
on and as a compensatory chamber  
of the stria vascularis may render the  
media anoxic, leading to a progressive  
ease in sodium and decrease in potassium  
n the endolymph, altering the endolymph  
ntial from approximately +100 mV to  
mV (Bosher & Warren, 1968), and per-  
s leading to hydrops. There may be a criti-  
level of endolymph potential at which the  
ae of the semicircular canals lose their  
ing neural function causing an attack of  
nal vertigo. Distension of the scala  
could become so great as to rupture  
ssner's membrane, causing an ion inter-  
nge and sudden alteration in the endo-  
ph potential. This would result in a more  
longed attack of vertigo. Unfortunately,  
nal studies gave conflicting results in dif-  
species and there is no animal model of  
s disorder, although endolymphatic  
s, with a low frequency fluctuating  
ng loss, has been demonstrated in me-  
cal cochlear models by Tonndorf (1968).

## GLYCEROL DEHYDRATION TEST

a trivalent alcohol (1,2,3, propane-  
l) given in high doses is not metabolized  
etely and is an osmotic diuretic, being  
in the urine. Klockhoff & Lindblom  
) used glycerol dehydration to produce  
icant hearing threshold shifts in cases of  
re's disease with fluctuating hearing

loss. They presumed that the osmotic effects  
of the glycerol reduced the endolymphatic  
hydrops and intra labyrinthine pressure and  
this was confirmed by Bosher & Warren  
(1971). Klockhoff & Lindblom employed 1.5 g/  
kg body weight of glycerol, although more re-  
cently they have used 1.2 g/kg. Originally, in-  
creases in the pure tone threshold of 5 dB  
were taken as significant, but more recently, a  
positive result has been taken as at least a  
10 dB improvement in three adjacent octave  
bands, or speech discrimination improvement  
exceeding 12% (Angelborg et al., 1973). No ef-  
fect was seen in more advanced Meniere's  
cases where a non fluctuating flat loss was  
present, or in sensorineural deafness of less  
specific types (Klockhoff & Lindblom, 1966).  
Morrison (1975), however, observed that in  
patients with well established Meniere's dis-  
order there may be a post glycerol improve-  
ment in speech discrimination in the absence  
of a pure tone threshold change. In a small  
proportion of patients there may even be an  
unexplained deterioration in the pure tone  
threshold or in speech discrimination in re-  
sponse to glycerol dehydration (Klockhoff,  
1975).

More recently Snyder (1971), confirmed  
Klockhoff's findings but felt a 15 dB increase  
in the pure tone threshold was more likely to  
be significant. He found that the changes were  
more marked in the low tones and also noted a  
significant increase in the speech discrimina-  
tion. Shea (quoted by Klockhoff, 1975) sug-  
gested that a good response to glycerol de-  
hydration was an indicator of a better prog-  
nosis in endolymphatic sac surgery.

This paper explains the role of continuous  
transtympanic electrocochleographic record-  
ing during glycerol dehydration in studying  
the electrophysiological changes in the  
cochlea.

## METHOD

Transtympanic electrocochleography, as de-  
scribed by Gibson et al. (1967), was employed.  
Adult patients were tested without sedation.

using the method of iontophoresis to anaesthetise the tympanic membrane (Ramsden et al, 1977)

Prior to the electrocochleography, blood and urine samples were collected for osmolality estimations. A pure tone audiogram and an assessment of speech discrimination were also carried out. All patients were starved from midnight for 12 hours before the test.

The electrodes were positioned with the patient lying comfortably in the supine position. A pre glycerol recording was carefully made and particular attention paid to electrode contact and the consistency of the response. Iced, lemon flavoured glycerol, 12 g/kg body weight, was given to the patient via a pliable straw so that the fluid could be taken without moving the head position. The immediate post glycerol response was then checked and full recordings to clicks and frequency specific sine waves at 1 and 2 hours post glycerol were made. The patients were also monitored at more frequent time intervals. In 2 patients the recordings were terminated at 1½ hours and at 1 hour respectively, because the patients became uncomfortable lying in one position for so long.

### *apparatus*

The Medelec Amplaid Mk III ERA equipment was used. This apparatus is simple to operate and is suitable for clinical purposes. The detailed specifications may be obtained on request from the manufacturers. In this study, the calculations in the tables and appendix are based on the responses obtained using acoustic clicks of 100 µs electrical duration. Spectral analysis of the click produced from the earphone showed that it contained a wide spectrum of frequencies with its maximal acoustic energy at 3 kHz. Pure tone bursts of 2-8 sine waves at specific audiometric frequencies were also employed as stimuli.

The stimuli were presented whenever possible, from a mu metal screened earphone which was attached magnetically to a special ring support placed around the ear. Occa-

sionally, the transducer was a loudspeaker positioned 50 cm from the test ear but this situation was avoided whenever possible since the test chamber was not anechoic. The bandpass of the differential amplifier was 3.2 Hz-3.2 kHz. The calibration of all signals was performed biologically to correspond to ISO threshold values and is expressed as decibels hearing level (dB HL).

### *Identification of the responses*

The response obtained using trans tympanic ECoChG is a complex mixture of several different bio-electric potentials. Each of these separate components was identified using the following techniques.

The action potential (AP) and the summating potential (SP) are direct responses whilst cochlear microphonic (CM) is an alternating potential. To isolate the action and direct responses, the number of stimuli are delivered in one phase (initially up going) and half the stimuli are delivered in the exact opposite phase (initially down going), and the responses to each are addressed by multiplexing to separate memory stores. Store A contains the AP and CM to one phase of stimulation and Store B contains the AP, SP and CM to the opposite phase of stimulation. When the stores A and B are added (A+B), the AP and SP are displayed with the CM suppressed. When using higher audiometric frequencies (above 1 kHz) this phase reversal makes little difference; the averaged AP waveform but at lower frequencies it leads to distortion as each individual nerve impulse is initiated by the upward (towards scala media) movement of the basilar membrane (Elberling & Salvi, 1971).

The CM may be isolated if the stores A and B are subtracted (A-B). This CM is not inverted as it is the averaged CM to one phase of stimulation added to the inverted averaged CM to the other phase of stimulation. It does not reflect the actual CM to one phase of stimulation alone and so CM distortion present could be overlooked. Recently

method does lead to the suppression of the AP waveforms, especially at the frequencies and to complete suppression of the SP

SP may be identified due to its lack of habituation. The d.c. responses are first obtained by using alternating stimuli of opposite phases (suppressing the CM) at a rate of 100 per second and the results are addressed to memory store A, under these circumstances the AP is displayed at its maximum amplitude together with the SP. Next, the responses are obtained using the same stimuli at a rate of 100 per second and they are addressed to memory store B, this time the AP shows the effects of adaptation (the exact degree of adaptation depends mostly on the stimulus interval, but using the click stimuli described at a rate of 100 per second, the AP is reduced to about one third of its true amplitude) whilst the SP component is unaffected.

Thus when the averaged responses in each memory store are subtracted (A-B), the AP is played out at about two-thirds of its maximum amplitude whilst the SP component is superimposed.

It is quite simple under these circumstances to judge the extent to which the SP, or similar potential, is affecting the SP/AP form. However, the AP waveform obtained after subtraction is distorted under conditions since at stimulus repetition of 100 per second, the latency and width of the AP are also affected (Eggermont & Durrant 1973) so subtraction of the memory leads to some differentiation of the AP.

In this series of patients the AP and CM responses were noted throughout each recording. Particular attention was paid to the AP waveform since this has been shown to be widened in cases of Meniere's disorder (Gibson & Beagley, 1976). This widening has been found to be due to an enhanced SP (or similar) component. It has been recently suggested that in general patients with an enhanced SP waveform benefitted more from

sacculus surgery than those with a more normal waveform (Gibson et al., 1976).

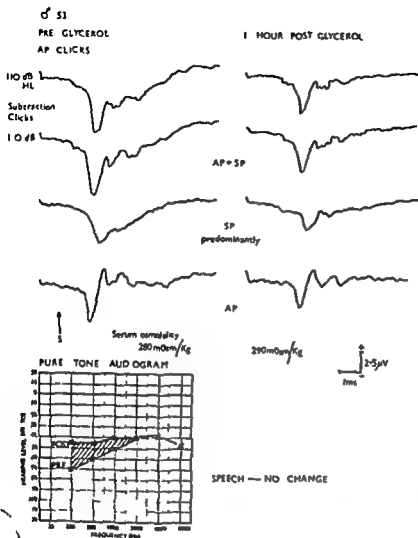
### *The summing potential*

In 1950 Davis, Fernandez, McAuliffe and Bekesy independently noted that a tonal stimulus can elicit a d.c. potential change in the normal cochlea at high stimulus intensities and since then the SP has been the subject of a great deal of research. The SP is a multi-component response representing the sum of various non-linearities occurring within the cochlea. The exact sources are still a matter of conjecture. Whitfield (1965) showed that a major component of the SP was derived from an asymmetry in the mechanism that produces the cochlear microphonic.

The non-linearity producing an increased negative SP in endolymphatic hydrops, may be due to displacement of the cochlear partition and thus asymmetry in the mechano-electric phenomena associated with hair cell stimulation. Durrant & Dallos (1974) have shown that mechanical displacement of the basilar membrane alters the SP recorded with intra cochlear electrodes. When the basilar membrane is displaced towards the scala media by raising the pressure in the scala tympani, the negative SP decreases in amplitude and may even change its polarity. Conversely, when the basilar membrane is displaced towards the scala tympani as would occur in endolymphatic hydrops the negative SP is enhanced. Similar results have been obtained after biasing the basilar membrane with low frequency sound and concurrently recording the SP using a transient high frequency stimulus (Durrant & Dallos 1974). An increase in the endolymphatic potential also accentuates the negative SP (Durrant & Gans, 1975).

### *Patient selection*

Eighteen patients, attending the London Hospital, were assessed using transtympanic electrocochleography during glycerol dehydration. All patients were felt, clinically, to be suffering from Meniere's disorder. The disease



**Fig 1** Marked decrease in the sensitive SP observed 1 hour after administration of glycerol. The shaded area in the pure tone audiogram represents the increase in threshold ( $S$  = stimulus onset).

was bilateral in 2 patients (11%). Audiometric findings indicated a recruiting hearing loss and the Hallpike Fitzgerald caloric test usually revealed a variable degree of paresis. It was evident that the certainty by which the diagnosis had been reached varied considerably. Four patients (22%) had all the classical manifestations of early Meniere's disease including a fluctuating low frequency hearing loss. It should be stated however that it is often difficult to determine whether the hearing loss is fluctuating. The patient may not notice if the variation in hearing is small and regular estimations of the pure tone threshold may fail to show any convincing change. Out of the 18 patients, 3 were excluded due to an unreliable

electrodochleographic response post caused by poor electrode contact. Two ther patients were excluded because change in the plasma osmolality, following hydration was less than 10 mOsm/kg. Ten patients were included in this series mean change in plasma osmolality was mOsm/kg with a range of 10-40 mOsm/kg.

**Table I**  
13 cars were tested

*[Faint handwritten notes at the bottom of the page]*

Q 46

PRE GLYCEROL

1 HOUR POST GLYCEROL

100 dB 21 ne waves 2 kHz - Subtraction technique  
HL

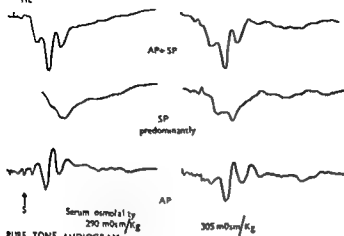


Fig 2 A smaller decrease in the negative SP following glycerol dehydration than in Fig 1. The change in the width of the SP is more noticeable than the change in the amplitude.

## RESULTS

### Changes in pure tone threshold and discrimination following glycerol dehydration

Patients were regarded as having had a significant change if the pure tone threshold (PTA) rose by 10 dB or greater at two or more isometric frequencies. A significant change in speech discrimination was taken as an improvement of 10% or more in the maximum discrimination score.

### Electrocochleography

The variation in the amplitude of the AP and CM occurred during the two-hour test period in some of the patients. This was possibly due to alteration in electrode contact. It was

therefore felt that under these circumstances conclusions drawn from changes in the amplitude of the AP and SP should be guarded. However, the width and amplitude of the SP was easily assessed and indeed alteration of the SP was seen to modify the widened AP/SP complex considerably. The negative SP was seen to decrease during glycerol dehydration.

In 4 patients the SP decreased maximally at 1 hour and after 2 hours the negative SP had increased again (see Fig 4).

Table II

11 ears were tested

9 ears	definite decrease in negative SP
( $>0.5 \mu V$ )	-69.2%
2 ears	probable decrease in negative SP
( $<0.5 \mu V$ )	-15.4%
2 ears	no change in the SP



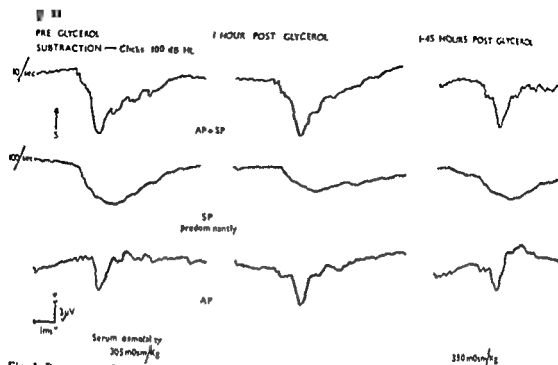


Fig 3 Progressive decrease in the negative SP up to 1.45 hours post glycerol

## DISCUSSION AND CONCLUSIONS

The striking feature of this study is the marked decrease in the negative SP during glycerol dehydration. It would seem possible that a decrease in the endolymphatic hydrops pro-

duces a movement of the basilar membrane towards the scala vestibuli thus decreasing asymmetry in the mechano-electric phenomena associated with the hair cells. Alternatively the dehydration may decrease

THE 9 EARS WHICH SHOWED A DEFINITE

DECREASE (305  $\mu$ V) IN THE SP AFTER

GLYCEROL DEHYDRATION & CHANGED

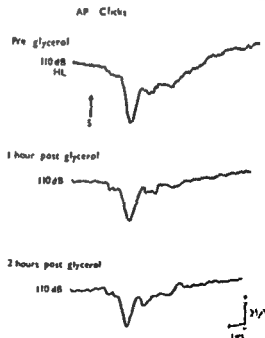
MAXIMALLY AT 1 HOUR AND AFTER

TWO HOURS THE SP HAD INCREASED

AGAIN

Fig 4

4 JUL 1985



olymphatic potential primarily, leading to creased negative SP. In 4 out of the 7 patients where the observed decrease in the negative SP took place, the change was maximal at 1 hour and thereafter the SP increased. In many patients with Meniere's disorder there may be no evidence of a fluctuating low hearing loss and no change in the pure tone threshold or in speech discrimination following glycerol dehydration. Yet Morrison (1975), has shown that, even in this group, ear surgery is often beneficial. Electrocochleography, however, appears to be a more sensitive indicator of changes occurring in the cochlea secondary to glycerol dehydration than subjective tests. All 13 patients tested have undergone endolymphatic sac surgery but are only in the early postoperative period. It would not be justified to classify these as successes or failures at this stage and the patients are undergoing prolonged follow-up, since long-term spontaneous remissions may occur. It is to be hoped that an observed decrease in the negative SP during glycerol dehydration may prove to be a useful pointer to the prognosis of endolymphatic sac surgery.

# ZUSAMMENFASSUNG

Zehn (13) Patienten mit der Mènièr'schen Störung wurden bei Anwendung einer Glycerol Dehydratation mit Hilfe von transtympanalen Elektrocochleogrammen getestet. Der häufigste Befund war eine Verminderung des negativen Summierungspotentials. Es stellte sich dabei heraus, dass dies ein feinerer Indikator für Veränderungen der Schnecke war als Rein-Ton Audiometrie und Sprachdiskrimination. Es wird im folgenden der Versuch unternommen, eine pathophysiologische Erklärung dieser Beobachtung anzubieten und die Möglichkeit der Nutzenmachung dieser Verminderung des negativen Summierungspotentials als ein Hinweis zur Prognose endolymphatischer Sackchirurgie wird diskutiert.

# REFERENCES

- Isborg C, Klockhoff I & Stahle J 1973 Serum osmolality in patients with Meniere's disease. *Acta Otolaryngol* 76: 450.  
S K & Warren R L 1968 Observations on the

- electrochemistry of the cochlear endolymph of the rat: a quantitative study of its electrical potential and ionic composition as determined by means of flame spectrophotometry. *Proc Roy Soc Biol* 171: 227.  
— 1971 A study of the electrochemistry and osmotic relationships of the cochlear fluids in the neonatal rat at the time of the development of the endocochlear potential. *J Physiol* 212: 739.  
Durrant J D & Dallos P 1974 Modification of DIF summing potential components by stimulus biasing. *J Acoust Soc Am* 56: 562.  
Durrant J D & Gans B 1975 Biasing of the summing potentials. *Acta Otolaryngol* (Stockh) 80: 13.  
Eggermont J J & Spoer A 1973 Cochlear adaptation in guinea pigs. *Audiology* 12: 193.  
Elberling C & Salomon G 1971 Electrical potentials from the inner ear in man in response to transient sounds generated in closed acoustic systems. *Rev Laryngol Suppl* 691.  
Gibson W P R & Beagley H A 1976 Transtympanic electrocochleography in the investigation of retrocochlear disorders. *Rev Laryngol* 97: Suppl. 53.  
Gibson W P R, Moffat D A & Ramsden R T 1977 Clinical electrocochleography in the diagnosis and management of Meniere's disorder. *Audiology* 16: 389.  
Hallpike P S & Cairns H 1938 Observations on the pathology of Meniere's syndrome. *J Laryngol Otol* 53: 625.  
Klockhoff I & Lindblom U 1966 Endolymphatic hydrops. In: Morrison A W (ed) *Endolymphatic Hydrops in Management of Sensorineural Deafness* (ed A W Morrison) chapter 5, p. 158. Butterworth & Co Ltd, London.  
Ramsden R T, Gibson W P R & Moffat D A 1977 Endolymphatic hydrops: a study of its symptomatology and pathology. *Laryngoscope* 87: 651.  
Snyder J M 1971 Changes in hearing associated with endolymphatic hydrops. *Laryngoscope* 81: 102-106.  
Whitfield J C & Ross H F 1965 Cochlear microphonic and summing potentials and the outputs of individual hair cell generators. *J Acoust Soc Am* 38: 126.  
D A Moffat Bsc FRCS  
Dept of Otolaryngology  
The London Hospital  
Whitechapel  
London E1  
Great Britain

## Appendix

Patient's initials	Change in serum osmolality (mOsm/kg)	Change in PTA (dB)	Change in speech discrimination (%)	Electrocochleography	
				AP/SP width (ms)	decrease in SP ( $\mu$ V)
S N	15	Nil	Nil	2.9	0.6
T I	10	↑ 10	↑ 20	2.4	0.6
I R	15	↑ 10	Nil	4.0	0.5
D C	12	Nil	Nil	2.9	0.55
A M	15	Nil	Nil	7.8	Nil
M B	18	Nil	↑ 24	0.75	0.4
A M	40	Nil	Nil	2.5	Nil
E K	15	↑ 10	↑ 10	3.75	0.5
D O	12	↓ 10	Nil	3.2	0.2
E L	25	Nil	Nil	4.0	0.6
D W	25	Nil	Nil	2.9	0.8
C T	10	↑ 30	Nil	5.9	1.0
V N	15	Nil	Nil	2.5	0.5

Decrease in the negative summing potential (SP) refers to the maximum observed decrease in the amplitude from the baseline in micro-volts using the subtraction technique as described in the text

## COCHLEAR FREQUENCY SHARPENING—A NEW SYNTHESIS

G A Manley

*From the Biology Department McGill University Montreal Quebec Canada*

(Received January 29 1977)

Recent evidence indicates a substantial difference in sharpness of tuning between basilar membrane mechanics and primary neuron responses in mammals. This paper describes a new qualitative model for a sharpening mechanism. It is suggested that the inner hair cells are sensitive to displacement changes in scala media induced by sound stimuli and that these changes can suppress neuron activity in a frequency-dependent way. The model explains sharpening of both of neural tuning curves, the shape of the low-frequency part of the tuning curves and is also compatible with other phenomena such as two-tone inhibition and the effect of electrical polarization of the basilar membrane.

This paper summarizes data defining the problem of frequency sharpening in the mammalian cochlea and which place profound constraints on models of sharpening mechanisms. A new synthesis, which is particularly suitable for experimental analysis, is presented and integrated with these data.

Békésy (1960) measured the frequency selectivity of different basilar membrane locations in various species by determining the sound pressure levels (SPL's) necessary to produce a certain displacement over a range of frequencies. More recently, similar responses in the guinea pig (Johnstone et al., 1970; Wilson & Johnstone, 1975) and squirrel monkey (Robles & Robles, 1974) have been measured at higher frequency locations in the cochlea at much lower SPL's. The new data are in good agreement with those of Békésy, but the sharpness of tuning is higher at these frequencies. Basilar membrane displacement

for constant SPL on the low frequency side of the best frequency falls off initially at less than 5 dB/octave. On the high frequency side, displacement falls off at about 130 dB/octave (Wilson & Johnstone, 1975), (Fig. 1).

Primary auditory neurone selectivity has been measured in the eighth nerve (Evans, 1972; Geisler et al., 1974; Kiang & Moxon, 1975) and spiral ganglion (Robertson & Manley, 1974) of mammals, including species used for basilar membrane determinations. These single neurone response patterns are remarkably consistent across species and, in animals in good physiological condition, their frequency selectivity is higher than that of the basilar membrane (Evans, 1974; Robertson & Manley, 1974). Even if it is assumed that cochlear hair cell responses are more related to basilar membrane velocity than to displacement, the differences are still large (Fig. 1). Typically, low frequency slopes of neurone tuning curves are more than 100 dB/octave near the most sensitive, or characteristic frequency (CF). On the high frequency side, slopes are commonly several hundred dB/octave (Fig. 1).

A saturating non-linearity found on the squirrel monkey basilar membrane (Rhode & Robles, 1974) at higher sound intensities does not appear to be a general phenomenon, as it

Supported by the Canadian National Research Council

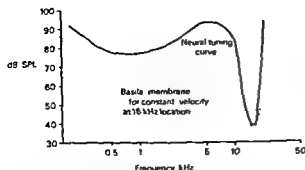


Fig. 1. A comparison of the mechanical frequency response of the 16 kHz location in the guinea pig cochlea and the frequency selectivity of a single neurone with CF 16 kHz on the same axes. The mechanical response is plotted as the sound pressure level necessary to produce a constant velocity in the 16 kHz location. Its position on the intensity scale is arbitrarily adjusted so that the most sensitive response is at the same level as the neural CF threshold. Because the most complete tuning curves are from the cat, the neural data here are from Liang & Moxon (1975). Deviations from guinea pig tuning curves are unlikely to be large, but may be significant at low frequencies. The dB differences between these curves are plotted in Fig. 2B.

did not appear in the guinea pig (Wilson & Johnstone, 1975).

### The second filter

Recent evidence confirms that in normally operating cochleas a fundamental difference in selectivity does exist between the mechanical and neural responses (Evans & Wilson, 1975; Geisler et al., 1974). Some of the models for a 'second filter' which creates this sharpening effect, particularly those models requiring complex mechanical transformations in the organ of Corti, are difficult to test experimentally. While many investigators favour some kind of non-classical inhibitory interaction between fibres innervating inner hair cells (IHC's) and outer hair cells (OHC's) (Evans, 1975; Lynn & Sayers, 1970; Zwislocki, 1975), experimental tests have many methodological difficulties. Current techniques which aim to 'selectively' eliminate OHC's from certain areas of the cochlea (e.g. ototoxic drugs, Ylikoski et al., 1974) are not sufficiently selective (Manley, 1975). It is not certain that nerve recordings from IHC only regions (Zwislocki, 1975) originate from nor-

mal IHC's, for response changes could be due to lack of OHC interaction or to IHC or OHC damage which is hard to detect, or both. Electron microscopy reveals changes in hair cells which look normal with light microscopy (Ylikoski et al., 1974). Damaged hair cells do not always disappear (Ryan & Dallos, 1974) and can continue to be present and presumably abnormally functioning even months after damage (Spoendlin, 1976). Disarrangement of the sensory hairs of IHC's appears only in toxic degeneration, the functional significance of which is unknown (Ylikoski et al., 1974). Zwislocki (1975) obtained abnormal responses from hair cell regions which looked normal in their surface preparations. Even in the whole organ of Corti appears normal, loss can be seen (Pinheiro et al., 1973). Fibre activity recorded from ototoxic damaged cochleas is very hard to interpret. None of the various theories of cochlear sharpening mechanisms has thus been the subject of definitive experimental verification. Ideally, models should be testable in cochleas with an intact complement of hair cells with little interference.

### Constraints on the model

I summarise here the features of recent models which a model must either try to explain or be compatible with. Some of these details, based on more solid evidence than others, for the present purpose will be treated as assumptions.

### Hair cells

1. Intracellular response potentials in the inner ear (Flock et al., 1973) and basilar (Weiss et al., 1974) hair cells are small, usually less than 3 mV well above threshold, compared with most other sensory cells. The secretory membranes of some hair cells are thus very sensitive to potential differences.
2. Recent findings indicate that the production of cochlear potentials (CM) and summating potentials (SP)

proportion to their relative number (Dallos et al., 1972a, b)

Stereocilia of IHC's are probably not attached to the tectorial membrane and thus will be stimulated by fluid velocity in the sub-tectorial space. While IHC microphonic is proportional to basilar membrane velocity, OHC microphonic is proportional to its displacement (Dallos, 1973). OHC stereocilia are firmly inserted in the tectorial membrane (Hohmann, 1974, 1976; Kimura, 1966; Kosaka et al., 1971; Lim, 1972; Ross, 1974). For IHC's, attachment varies, ranging from a type of attachment similar to that of OHC's (Iurato, 1967; Lim, 1974), IHC attachment in some parts of the cochlea of only certain species (Hoshino, 1976), to no connection at all (Kimura, 1966; Liberman et al., 1971).

During ontogeny, the tectorial membrane separates more and more from the hair cells (Hoshino, 1974). The available evidence, taken together, indicates that the separation goes further in the IHC area than in the OHC area. In cats, IHC's of the basal turn have impressions in the tectorial membrane, while shallow imprints show that even in this region any possible connection is less firm than that of OHC's (Hoshino, 1976).

In the guinea pig, there are no impressions on the IHC's. Even the indistinct imprints in the cat's cochlea may be only remnants of ontogenetic processes.

IHC and OHC organelles show conspicuous differences in size, arrangement and distribution (Spoendlin, 1975). IHC afferent synaptic structures appear to conform more to the typical chemical synapse arrangement than do the afferent synapses of OHC's (Spoendlin, 1975).

#### Innervation

More than 90% of the afferent innervation terminates on IHC's, apparently with most axons only contacting a single IHC. Although OHC's make up about 80% of the receptor cell population, they receive less than 10% of the afferent innervation (Spoendlin, 1975).

2 There appears to be no anatomical evidence for pathways which could obviously mediate interactions between OHC and IHC afferents. Classical lateral inhibition is ruled out by this anatomical evidence and by the fact that the tuning properties of the afferent system do not require time (e.g., synaptic delays) to develop (de Boer, 1969; Evans, 1975b; Møller, 1972).

3 There are conspicuous metabolic differences between IHC and OHC dendrites (Spoendlin, 1975). IHC dendrites are acutely sensitive to anoxia, whereas OHC dendrites are unaffected by brief exposure (Spoendlin, 1973). Similarly, sectioning of the auditory nerve leads to degeneration of the IHC afferents in a few weeks, whereas OHC afferents survive more than a year (Spoendlin, 1973, 1975).

#### Afferent fibre activity

1 Microelectrode recordings from the eighth nerve (Evans, 1972; Kiang & Moxon, 1975) or spiral ganglion (Robertson & Manley, 1974; Manley & Robertson, 1976) sample a homogeneous population of cells. For example, in any one location in the spiral ganglion of an individual guinea pig (fibres of identical CF), the range of thresholds is only  $6.7 \pm 3.9$  dB (Robertson, 1975). Either the adequate stimulus for the outer spiral fibres has not been found (assuming they do not show spontaneous activity) or the primary function of OHC afferents is not the transmission of auditory input to the central nervous system.

2 While the crossed olivo-cochlear bundle (COCB, one component of the efferent pathway) appears to have most of its inhibitory terminals on OHC somata (Spoendlin, 1973), its activation affects all primary afferent fibre activity in an inhibitory fashion (Wiederhold & Kiang, 1970).

#### Pre neural sharpening

One possibility for a sharpening mechanism which has not received much attention is pre neural sharpening via interactions between

hair cells directly through the extracellular space of scala media. Earlier model stimulations (Strelhoff, 1973) have demonstrated that shunting of current by nearby hair cells must occur in the cochlea, and such an interaction could affect neural activity under certain conditions. This idea is similar to the notion that hair cells might be affected by small potential changes in the endolymphatic space, produced by the activity of neighbouring hair cells.

I believe the features of the recent data summarized above are best accounted for by the following general scheme. All afferent fibre activity is recorded from IHC afferents. OHC afferents are silent but necessary for OHC maintenance. OHC's are specialized as variable channels for current to flow out of scala media in quantities sufficient to affect the resting potential level of scala media (the endocochlear potential or EP). This flow of current occurs, or is increased, during sound stimulation or during COCB activation. IHC's are sensitive to both the fluid velocity in the sub tectonal space and to d.c. electrical changes induced by OHC activity (negative summing potentials, or  $-SP$  are of most interest here, reaching values of 3–5 mV in scala media in the normal recording locations) (Dallos et al., 1972b; Honrubia & Ward, 1969a). This  $-SP$  suppresses sound evoked activity in IHC's. Whether or not the output of an IHC synapse changes is assumed to depend both on the mechanical stimulus and the size of the  $-SP$  (Manley, 1976).

It is not unreasonable to suggest that the hair cells producing the neural output (the IHC's) might be sensitive to small changes in their electrical environment as vertebrate hair cells (neuromasts) and teleost electroreceptors have a common origin in mechanoreceptive lateral line cells of fish (Bennett, 1970). Both mechanical and electrical sensitivity of neuromast receptors (Suga, 1967) are considerably enhanced by high potassium ion concentrations over the hair cell (Hashimoto & Katsuki, 1972), as in the endolymph of the cochlea. Even without such high potassium levels

neuromast derivatives can have high activity. Tonic electroreceptors, for example, are sensitive to external d.c. potential changes much smaller than the typical receptor potentials of receptor cells. A swing of electric voltage from  $-1$  mV to  $+1$  mV is sufficient to take tonic electroreceptor afferent fibres of *Gymnotus* from 0 to 300 impulses per second over their entire dynamic range (Bennett, 1970).

To illustrate how such an electrical sensitivity on the part of IHC's could shape neural tuning curves, consider an experiment to determine the threshold response tuning curve for a neurone in the guinea pig and nerve most sensitive to a tone of frequency 16 kHz (the CF). At the extremely low SPL necessary for a response at the CF, the  $-SP$  at this CF location would be extremely small (Honrubia & Ward, 1969a). This  $-SP$  would have little or no suppressive effect on the IHC and thus the low mechanical threshold of the cell is not affected. When stimulus frequency is dropped an octave to 8 kHz, SPL would have to be raised 3–4 dB (Honrubia, 1974) to compensate for the smaller displacement in the CF location to this new tone. However if the IHC's are velocity sensitive, SPL would have to be raised an additional 10 dB to produce the same velocity in the CF location (I assume that the velocity necessary to induce a threshold conductivity increase in the IHC is achieved at CF at the same SPL as the displacement necessary to induce a threshold conductivity change in OHC's).

The new travelling wave envelope produced by the 8 kHz tone peaks about 2.5 mm anteriorward of the CF location (Wilson, 1974) because of the 9–10 dB increase in SPL. It relates a much larger area of cells. At the location, while the velocity of the basilar membrane again matches that of the CF threshold, the OHC's are being stimulated at a level above that of the CF frequency and threshold. They therefore undergo a larger resistive change and the  $-SP$  produced by all the cells is much larger. The model suggests the final  $-SP$  in the CF location produced

3 kHz tone is large enough to suppress the response to the mechanical stimulus. The must, therefore, be raised in intensity. Of course a higher SPL will produce not only greater mechanical stimulation but also a larger -SP.

There is thus an antagonism between increasing mechanical stimulus and the increasing -SP. The point at which the suppression becomes insufficient to prevent increased synaptic output is the new threshold. The same reasoning would apply to frequencies of stimulation higher than the CF. Here, although matching velocity in the CF region is attained at the matching displacement, the slope of the tuning curve of the basilar membrane is so steep that OHC's in the new peak location are receiving very large inputs and therefore producing a large -SP in the nearby CF region.

If the lateral spread of suppression is limited to the space constant of scala media (1.5 to 2 mm, Strelhoff, 1973, or about 10 dB/octave),

on the low frequency side of the tuning curve the suppressive effect tails off when the peak of the travelling wave is very distant from the CF location. On the high side of the tuning curve, the peak locations never become too far away to be effective. The model thus accounts for sharper neural tuning curves on both the high and the low frequency sides of the tuning curves and for the progressive reduction of the effect at low frequencies (the so-called 'tail' of the tuning curve, Kiang & Moxon, 1975). When the animal is made hypoxic, or otherwise has its auditory sensitivity artificially depressed, the tuning curve would, on this hypothesis, become broader (Robertson & Manley, 1974). A possible additional consequence of this antagonism might be a broadening of the dynamic range of IHC input. Under hypoxic conditions also, dynamic ranges at CF are reduced to half their normal value (Evans, 1975, Robertson, 1975). If the above scheme is valid as a working hypothesis, there should be a good correlation between the size of the -SP in the CF location and the neurone threshold for any given frequency and the discrepancy at that frequency between

the mechanical velocity curve for the CF location and the neural tuning curve. From my own (unpublished) data and from published accounts (Dallos et al., 1972*b*, Honrubia & Ward, 1969*a*, Manley & Johnstone, 1974) a good estimate can be made of the -SP present in, for example, a 16 kHz CF location at the frequencies and intensities which form a threshold curve for a neuron in that location (Fig. 2). By normalizing the frequencies I compare directly the -SP at a lower frequency location, such as for a CF 2.8 kHz unit from Evans (1972). The resemblance between the two functions is good, the largest -SP's being generated near normalised frequency 0.6, or less than an octave below the CF. This corresponds well to the frequency at which the low frequency slope of the tuning curve of a neurone becomes less steep. Below this frequency, the discrepancy between mechanical and neural curves also decreases (Fig. 2).

### Two tone suppression

The idea that -SP present at the CF location but mainly produced in another location suppresses activity in the CF location is relevant to the phenomenon of two-tone suppression or inhibition. It is well known (Arthur et al., 1971, Sachs, 1969, Sachs & Kiang, 1968) that when a tone at the CF and a little above threshold stimulates a neurone, the response can be suppressed by a tone of higher or lower frequency which, when presented alone, is not in most cases loud enough to stimulate the cell. According to my model, the -SP present at the CF location and generated by the travelling wave of the second tone could suppress the response. Quantitative interpretation is complicated by the fact that both tones produce some mechanical input to the CF location and both produce some -SP. It is noteworthy that the sharpness of the tuning of the inhibitory areas and of the single tone excitatory areas increases with increasing CF (Sachs & Kiang, 1968), suggesting that the mechanisms of normal sharpening and two-tone suppression share common features. Kiang & Moxon



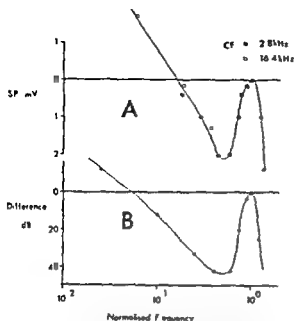


Fig. 2 (A) Size and polarity of SP produced in scala media by the frequencies and intensities normally used to obtain threshold responses from two neurones of different CF. The SP data for the tuning curve of the neurone with CF 16.4 kHz are for the appropriate location in scala media of the basal turn of the cochlea and for the neurone with CF 2.8 kHz are from scala media records from the second turn. The frequency of the CF's has been normalized to 10<sup>0</sup> and other frequencies are represented as fractional values of this. The curve is an eye fit to both sets of data points. (B) Difference between mechanical and neural curves of Fig. 1.

1975) show the suppressive effect of low frequency noise bands on high frequency tuning curves. These noise bands alone just produced activity in the unit for the lowest intensities used but suppressed parts of the single tone tuning curve. Fig. 3 presents a graph of this frequency dependent suppression for a unit of CF near 13 kHz as calculated from their data. The maximum effect of the noise is near the CF, where my hypothesis predicts that there is no pre-existing suppression. There is no effect about an octave below the CF where there is already a large existing suppression. These frequency-dependent effects are therefore highly compatible with this model.

The response of a neurone at CF and off CF are therefore seen as the result of different degrees of interactive forces. This is in keeping with the finding that the tips of tuning

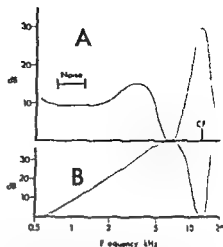


Fig. 3 (A) Diagram of the inhibitory effect. Portions of the tuning curve of a neurone of CF 13 kHz of a 500 Hz wide band of noise centred at 1 kHz was calculated from data of Kiang & Moxon (1974). The noise intensity was about 15 dB higher than that which alone just evoke a response from the unit. (B) Inhibitory effects for noise bands both more and less intense than similar shapes. This noise band produces a 10 dB reduction to CF tones but has no effect on responses to tones near 6-7 kHz. (B) Inhibitory function calculated from data of Kiang & Moxon (1974).

environment of the IHC's in the CF location. It is clear from (A) that the noise band has no effect on the tuning curve at a frequency where the inhibitory function is already at a large amount of suppression already existing. The maximum of this inhibitory function is placed at 40 dB to compare with Fig. 2B.

These curves behave differently than the tips of tuning curves of toxic drugs, high intensity sounds, COCB stimulation and low frequency masking. The tuning curves mostly in the tips (Kiang & Moxon 1975). In essence the tuning curve represents a response already partially masked by suppression, and the tips are therefore much less susceptible to interference.

### Efferent suppression

An additional value of this model is that it helps to understand the frequency-dependent effects of COCB stimulation. According to Green (1974a, b) the COCB could act via its interaction

increasing the OHC conductance, shunting it away from IHC's. Thus, the IHC threshold would be increased and the rate of firing moved to higher SPL's. In my model, COCB makes use of an existing suppressive mechanism for affecting IHC output by modifying its electrical environment. The meters normally used (Sachs & Kiang, 1961) to stimulate the COCB produce a slow EP of 3–4 mV. My model predicts this change would be suppressive near the CF but at off CF frequencies the effect would combine with pre-existing suppression in a complex way, as SP itself is affected by COCB modulation. Thus the effect should be maximal at the CF, as is the case (Wiederhold & Rug, 1970). Any explanation of COCB effect is complicated by the fact that a proportion of COCB fibres in some species form dendritic synapses on IHC radial afferents (Wiederhold, 1975).

#### *Official polarization of the cochlea*

Electrical polarization of the cochlea changes magnitude and even the sign of the EP and affects the other dependent potentials (Honrubia & Ward, 1969b). It also affects the activity of eighth nerve fibres (Konishi et al., 1970; Teas et al., 1970). Unfortunately, in the latter case, the magnitude of the EP and SP shifts produced by the polarizations were not assured. According to Honrubia & Ward (1969b) however, the current crossing the silar membrane under these conditions is less than one tenth of the total, so that the potential changes were probably not large. It is likely possible at this stage to see if the data is qualitatively compatible with my hypothesis. In the first place, if the imposed current flow opposes the normal outflow of ions from scala media through the hair cells, polarization could be expected to suppress the responses to tonal stimulation, and such is the case in 10% of the fibres. I will discuss the other 10% below. This is only true, if the tone used is at or near the CF. At other frequencies, such current increases tone evoked discharge rates

(Teas et al., 1970). This anomalous discharge increase is explained by my model by supposing that the dominant effect of the current for tones off the CF is to oppose the outflow from scala media of a current which is suppressing the fibre, i.e., to 'disinhibit' the fibre. There being no significant suppressive current from tones at the CF near threshold, the current then suppresses the unit's activity. With regard to the 10% of the units which increase their discharge at the CF, I suggest that this is due to an inaccurate estimate of the CF. Teas et al. (1970) state that due to the limitations of their equipment they did not measure accurate tuning curves.

#### *Comparative aspects*

Given that all neuromast derivatives have some degree of both mechanical and electrical sensitivity, it is reasonable to suppose that the generalities of the scheme outlined above apply also to the ears of non-mammalian vertebrates. Granted that mammals have greater specialization of both hair cell populations and innervation patterns, but the basic idea would apply to any spatial array of receptor cells which are influenced by potential changes or current flow changes induced by their neighbours. Thus some of the factors in this model might also be involved in the sharp tuning found in non-mammalian primary fibres, and the two-tone inhibitory phenomenon in birds (Sachs et al., 1974).

#### *Experimental tests*

This hypothesis is offered in the hope that it will stimulate both new experiments and formal quantitative analysis, taking more factors into detailed consideration. At the moment it seems that cochlear frequency sharpening can best be explained by a combination of something like the present hypothesis and some sort of differential coupling of the IHC cilia to basilar membrane motion at different frequencies (Duifhuis, 1976; Zwicker, 1976). Some relatively straightforward tests of the present hypothesis, looking at the effects of modifying

the electrical environment of the hair cells on tuning properties, would bring us closer to the solution of this interesting and important problem. Auditory masking experiments which have demonstrated longitudinal cochlear interactions probably do occur (Ryan & Dallos, 1975*b*) might also be re-examined in relation to the present hypothesis.

Finally, it should be noted that analogous cases of interaction via electrical lateral suppression have recently been described (I attempted in this paper to retain the term 'inhibition' for events mediated by special intercellular junctions, and suppression for a phenomenologically similar event mediated differently). Mutual suppression of different colour receptors of the locust eye has been shown (Shaw, 1975). Due to the pattern of extracellular resistances ions from strongly stimulated receptors flow out through weakly responding ones (with different colour sensitivity) and suppress their activity. Similar contrast enhancing mechanisms operate for polarized light sensitivity in the crayfish eye (Muller, 1973). As Shaw points out, this system can produce selective and mutual suppression without needing complex genetic programmes for the inhibitory wiring of neural afferents. This kind of lateral electrical suppression is a phenomenon whose importance in sensory arrays is only now beginning to gain recognition.

### NOTT ADDED IN PROOF

Two important papers have appeared since the completion of this manuscript. Russell and Sellick (1977*a*) demonstrated that the receptor potentials of the inner hair cells of guinea pigs are much larger than expected (up to 15 mV) and that these receptor potentials are just as sharply tuned as the nerve fibre responses. Although the latter finding is predicted by my model, measurements of impedance changes in hair cells during tonal stimulation (Russell and Sellick, 1977*b*) place important restric-

tions on the applicability of the model described here.

### ACKNOWLEDGEMENT

I thank R. R. Capranica, R. Chase, E. F. Evans, G. E. H. H. Levine, J. P. Wilson and J. Winkler for discussions, R. Funnell and J. Winkler for programming and L. Pawson for technical assistance.

### ZUSAMMENFASSUNG

Neue Ergebnisse weisen darauf hin, daß es einen erheblichen Unterschied in der Frequenzselektivität zwischen der Basalmembran und den primären inneren Haarzellen gibt. Diese Arbeit beschreibt ein theoretisches Modell, das die Verbesserung der Frequenzselektivität erklärt. Hierbei wird angenommen, daß die Haarzellen frequenzabhängig unterdrückt werden, daß diese Potentialänderungen die Aktivität der primären Haarzellen frequenzabhängig unterdrücken. Das Modell erklärt, wie im Vergleich zur Basalmembran beide Seiten der Schwellenkurven der primären Haarzellen steiler werden und berücksichtigt auch die Frequenzkompatibilität der Zweitonhemmung und den Aufbau von elektrischer Polarisierung an der Basalmembran.

### REFERENCES

- Arthur, R. M., Pfeiffer, R. & Supa, V. 1971. Properties of two-tone inhibition in primary auditory cortex. *J. Neurophysiol.* 34, 601-611.
- Bennett, M. V. L. 1970. Comparative physiology of the organs. *Ann. Rev. Physiol.* 32, 471-481.
- Boer, E. de. 1969. Reverse correlation of nerve impulses in the inner ear. *Proc. Konink. Akad. Wetensch. C* 72, 129-139.
- Dallos, P. 1973. Cochlear potentials and cochlear mechanics. In *Basic Mechanisms in Hearing* (Ed. Møller), pp. 335-376. Academic Press, New York.
- Dallos, P., Billone, M. C., Durrant, J. D., Wang, C. & Raynor, S. 1972*a*. Cochlear inner and outer hair cell functional differences. *Science* 177, 346-348.
- Dallos, P., Schoenly, Z. G. & Cheatham, M. A. 1972. Cochlear summating potentials: descriptive and functional studies. *Acta Otolaryngol. (Stockh.)* Suppl. 303.
- DuShane, H. 1976. Cochlear nonlinearity and its filter: possible mechanisms and implications. *J. Acoust. Soc. Am.* 59, 408-418.
- Dunn, R. A. 1975. Receptor synapses and their role in the cochlea of the cat. In *Proc. 1st Meet. Neurosci. Soc.* New York, p. 25.
- Evans, E. F. 1972. The frequency response and properties of single fibres in the guinea pig cochlea. *J. Physiol. (Lond.)* 229, 261-281.

- 974 Auditory frequency selectivity and the cochlear curve. In *Facts and Models in Hearing* (ed E Zwicker & E Terhardt) pp 118–129. Springer, New York.
- 975 Normal and abnormal functioning of the cochlear curve. *Symp Zool Soc Lond* 37: 133.
- 976 E F & Wilson J P 1975 Cochlear tuning properties: concurrent basilar membrane and single nerve fiber measurements. *Science* 190: 1218.
- 977 K Å, Jørgensen M & Russell I 1973 The physiology of individual hair cells and their synapses. In *Basic Mechanisms in Hearing* (ed A R Møller) pp 107–134. Academic Press, New York.
- 978 C D 1974a Hypothesis on the function of the crossed olivocochlear bundle. *J Acoust Soc Am* 56: 908.
- 979 C D 1974b Model of crossed olivocochlear bundle effects. *J Acoust Soc Am* 56: 1910.
- 980 C D, Rhode W S & Kennedy D T 1974 Responses to tonal stimuli of single auditory fibers and their relationship to basilar membrane motion in the squirrel monkey. *J Neurophysiol* 37: 1156.
- 981 Imoto T & Katsuki Y 1972 Enhancement of the mechanosensitivity of hair cells of the lateral line organs by environmental potassium ions. *J Acoust Soc Am* 52: 553.
- 982 Rubin V & Ward P 1969a Temporal and spatial distribution of the CM and SP of the cochlea. In *Frequency Analysis and Periodicity Detection in Hearing* (ed R Plomp & G F Smoorenburg) pp 94–106. Dordrecht, Netherlands.
- 983 Rubin V 1969b Dependence of the cochlear microphonics and the summing potential on the endocochlear potential. *J Acoust Soc Am* 46: 388.
- 984 Rubin V & T 1974 Relationship of the tectorial membrane to the organ of Corti: a scanning electron microscope study of cats and guinea pigs. *Arch Histol Jap* 37: 25.
- 985 1976 Attachment of the inner sensory cell hairs to the tectorial membrane: a scanning electron microscope study. *ORL* 38: 11.
- 986 T 1967 *Submicroscopic Structure of the Inner Ear*. Pergamon Press, Oxford.
- 987 Johnstone B M, Taylor M J & Boyle A J 1970 Mechanics of the guinea pig cochlea. *J Acoust Soc Am* 47: 504.
- 988 Ng N Y S & Moxon J C 1975 Tails of tuning curves of auditory nerve fibers. *J Acoust Soc Am* 55: 610.
- 989 Nara R S 1966 Hairs of the cochlear sensory cells and their attachment to the tectorial membrane. *Acta Otolaryngol* (Stockh) 61: 55.
- 990 Nishi T, Teas D C & Wernick J 1970 Effects of electrical current applied to cochlear partition on discharges in individual auditory nerve fibers. I. Prolonged direct current polarization. *J Acoust Soc Am* 47: 1519.
- 991 Naka, N, Tanaka T, Takiguchi Y, Ozeki Y & Takahara S 1971 Observation on the organ of Corti with the scanning electron microscope. *Acta Otolaryngol* (Stockh) 72: 377.
- 992 D J 1972 Fine morphology of the tectorial membrane. Its relationship to the organ of Corti. *Arch Otolaryngol* 96: 199.
- 993 Lindeman H H, Ades H W, Bredberg G & Engstrom H 1971 The sensory hairs and the tectorial membrane in the development of the cat's organ of Corti. *Acta Otolaryngol* (Stockh) 72: 229.
- 994 Lynn P A & Sayers M McA 1970 Cochlear innervation: signal processing and their relation to auditory time intensity effects. *J Acoust Soc Am* 47: 575.
- 995 Manley G A 1975 Function of cochlear hair cells. *Nature* 255: 657.
- 996 — 1976 Cochlear frequency sharpening—a new model. *J Acoust Soc Am* 59: Suppl 1: S31.
- 997 Manley G A & Robertson D 1976 Analysis of spontaneous activity of auditory neurons in the spiral ganglion of the guinea pig cochlea. *J Physiol* (Lond) 258: 323.
- 998 Manley J A & Johnstone B M 1974 A comparison of cochlear summating potentials in the bat and guinea pig, including temperature effects. *J Comp Physiol* 88: 43.
- 999 Møller A R 1972 Coding of sounds in lower levels of the auditory system. *Q Rev Biophys* 5: 59.
- 1000 Muller K J 1973 Photoreceptors in the crayfish compound eye: electrical interactions between cells as related to polarized light sensitivity. *J Physiol* (Lond) 232: 573.
- 1001 Pinheiro M, Jordan V & Luz G A 1973 The relationship between permanent threshold shift and the loss of hair cells in monkeys exposed to impulse noise. *Acta Otolaryngol* (Stockh) Suppl 312.
- 1002 Rhode W S & Robles L 1974 Evidence from Mossbauer experiments for nonlinear vibration in the cochlea. *J Acoust Soc Am* 55: 588.
- 1003 Robertson D 1975 *Studies of single neurone activity in the cochlear ganglion of the guinea pig*. Ph.D. Thesis, McGill University, Montreal.
- 1004 Robertson D & Manley G A 1974 Manipulation of frequency analysis in the cochlear ganglion of the guinea pig. *J Comp Physiol* 91: 363.
- 1005 Ross J 1974 The tectorial membrane of the rat. *Am J Anat* 139: 449.
- 1006 Russell I J & Sellick P 1977a Tuning properties of cochlear hair cells. *Nature* 267: 858.
- 1007 — 1977b The tuning properties of cochlear hair cells and Addendum. In *Psychophysics and Physiology of Hearing* (ed E F Evans & J P Wilson) pp 71–84. Academic Press, London.
- 1008 Ryan A & Dallos P 1975a Function of cochlear hair cells. reply. *Nature* 255: 657.
- 1009 — 1975b Mechanisms of auditory masking. *J Acoust Soc Am* 57: Suppl 1: p 41.
- 1010 Sachs M 1969 Stimulus-response relation for auditory nerve fibres: two tone stimuli. *J Acoust Soc Am* 45: 1025.
- 1011 Sachs M B & Kiang N Y 1968 Two-tone inhibition in auditory nerve fibers. *J Acoust Soc Am* 43: 1120.
- 1012 Sachs M 1974 Young E D & Lewis R 1974 Discharge patterns of single fibers in the pigeon auditory nerve. *Brain Res* 70: 431.
- 1013 Shaw S R 1975 Retinal resistance barriers and electrical lateral inhibition. *Nature* 255: 480.
- 1014 Spoendlin H 1973 The innervation of the cochlear receptor. In *Basic Mechanisms in Hearing* (ed A R Møller) pp 185–234. Academic Press, New York.

- 1973 Neuratomical basis of cochlear coding mechanisms *Audiology* 14, 383
- 1976 Anatomical changes following various noise exposures. In *Effects of Noise on Hearing* (ed D Henderson, R P Hamernick, D Dosanjh & J Mills) pp 69–90 Raven Press New York
- Strehloff, D 1973 A computer simulation of the generation and distribution of cochlear potentials *J Acoust Soc Am* 54, 620
- Suga, N 1967 Electrosensitivity of specialised and ordinary lateral line organs of the electric fish *Gymnotus carapo*. In *Lateral Line Detectors* (ed P Cahn), pp 395–409 Indiana Univ Press, Bloomington
- Teas, D C, Konishi, T & Wernick, J S 1970 Effects of electrical current applied to cochlear partition on discharges in individual auditory nerve fibres. II. Interaction of electrical polarization and acoustical stimulation *J Acoust Soc Am* 47, 1527
- Weiss, T F, Mulroy, M J & Altmann, D W 1974 Intracellular responses to acoustic clicks in the inner ear of the alligator lizard *J Acoust Soc Am* 55, 606
- Wiederhold, M L & Kiang, N Y-s 1970 Effects of electrical stimulation of the crossed olivo-cochlear bundle on single auditory-nerve fibers in the cat *J Acoust Soc Am* 49, 940
- Wilson, J P & Johnstone, J R 1975 Bulbar and middle-ear vibration in guinea pigs: a capacitance probe *J Acoust Soc Am* 57, 71
- Ylikoski, J, Wersäll, J & Björkroth B 1974 Cx studies on the cochlear pathology and hearing in guinea pigs after intoxication with ototoxic drugs *Acta Otolaryngol* (Stockh) Suppl 335
- Zwicker, E 1974 On a psychoacoustical equalizing curves. In *Facts and Models in Hearing* (ed E Zwicker & J Terhardt) pp 94–99 Springer, York
- Zwislocki, J J 1975 Phase opposition between outer hair cells and auditory sound analysis *Acta* 14, 443

G. A. Manley, Ph.D.  
 Institut für Elektroakustik  
 Technische Universität München  
 Arcisstr. 21  
 8000 München 2  
 Federal Republic of Germany

# RELATION BETWEEN THE WAVEFORM OF THE COCHLEAR WHOLE NERVE ACTION POTENTIAL AND ITS INTENSITY FUNCTION

J P Legoux, D C Teas,<sup>1</sup> H A Beagley<sup>2</sup> and M C Remond

*Laboratoire de Neurophysiologie Collège de France Paris France*

(Received May 15 1977)

*Abstract* The whole nerve action potential (AP) was recorded by intracochlear electrodes in the guinea pig. As well known in normal conditions the AP elicited by a burst displays negative and positive deflections. Inactivation of the central end of the nerve at the internal auditory meatus (IAM) by introduction of a few drops of KCl solution or by mechanical pressure, produces a change in the wave shape of the AP which is transformed into a single negative deflection. The relation of the amplitude of the monophasic AP to the intensity of the tone burst is monotonic in contrast to the classical two-slope relation observed in normal conditions. These results are interpreted by the disappearance of a positive component of the response produced at the IAM. The conclusion of the monophasic unit AP would explain the monotonic intensity function.

responses (AP), Teas et al (1962) presented a model of the AP to explain how diphasic neural elements of the population of fibres could combine in amplitude and phase as a travelling wave excites the elements successively, to produce the classical  $N_1$  and  $N_2$  deflections. The accuracy of this model was strongly supported by Legoux & Pierson (1974) who found that inactivation of the internal end of the nerve by KCl solution made the AP recorded by cochlear electrodes monophasic. They also observed that the intensity function of the AP was modified when the waveform of the AP was altered. These results were in agreement with the model of Teas et al.

This paper reports a series of experiments on the guinea pig which were devised to show that the contribution of two sources of AP at both ends of the meatus produces positive and negative waves which combine to produce various resultant waveforms. They show also that some of the classical characteristics of whole nerve intensity functions are altered by blocking transmission of nerve impulses at the internal auditory meatus (IAM).

<sup>1</sup> Dr Teas was a visiting scientist at Collège de France from the Institute for Advanced Study of the Communication Processes and the Department of Psychology, University of Florida, Gainesville, Florida 32611, USA.

<sup>2</sup> Dr Beagley was a visiting scientist from the Institute of Laryngology and Otology, 330 Gray's Inn Road, London WC1.

Davis et al (1952) gave an interpretation of the whole nerve action potential (AP) recorded by electrodes in contact or penetrating inside the guinea pig cochlea. They indicated that the source of the AP was localized at the level of the internal auditory meatus where the tightly myelinated fibres pass through the bone. They pointed out that because the nerve traverses an insulating bony tube, two physiological electrodes are constituted by the two openings of the tube. These anatomically determined electrodes represent the effective recording sites when the outputs from wires placed in scala tympani and scala vestibuli are summed and expressed against a remote grounding point on the animal. Since such physiological electrodes should produce diphasic neural

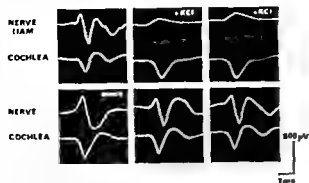


Fig. 1. Responses recorded from the auditory nerve (AP) and internal auditory meatus (IAM) in the guinea pig to a 7 kHz tone pip with rise time of 10 msec. The upper left panel shows control waveforms. The upper middle panel shows the response waveforms after application of KCl to the IAM region. Only a small positive deflection remains at IAM, probably due to voltage pick up from the cochlear side of the IAM. The (cochlear) AP has lost the positive peak and is broader than for the control response. Upon washing with saline, the response begins to recover as shown in the lower panels.

## METHOD

The APs were recorded from guinea pigs (350–400 g) anesthetized with ethylurethane. In some cases the animal was curarized and artificially ventilated. The recording electrodes were steel or copper wires 100 µm diameter. These electrodes were introduced into scala tympani and scala vestibuli in the basal turn and sometimes in the third turn, recording to the classical differential technique. Another electrode was usually introduced in the proximity of the nerve trunk at the internal auditory meatus (IAM) after opening the skull and removing part of the cerebellum.

To inactivate the central end of the nerve a technique similar to that used by Legoux & Pierson (1974) was applied: a few drops of a KCl solution was introduced (0.1 N) in the vicinity of the nerve. This procedure resulted in a decrease in the response under the IAM electrode. This effect was reversible and the IAM response recovered in a few minutes. The procedure could be repeated safely several times. In other cases, inactivation of the nerve at the IAM was produced by mechanical pressure exerted by a tiny cotton ball intro-

duced near the nerve trunk. In this case the effects could be very gradual but reversibility was sometimes incomplete.

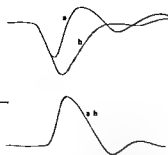
In most experiments the animal was held with an ear bar on one side and a screw into the external auditory meatus on the other. Clicks or tone bursts were provided by a speaker located 5 cm from the opening of the speculum. The responses were displayed on an oscilloscope and photographed. In many instances the APs were averaged to improve S/N ratio. A total of 40 guinea pigs were used in these experiments.

### *Contribution of the IAM Source to the AP Recorded by Differential Electrodes in the First Turn*

To evaluate the contribution of the IAM source to the AP recorded by the differential electrodes (their sum) in the first turn, the response was observed before and after inactivation of the IAM source by the KCl solution.

The electrode located in the vicinity of the internal auditory meatus recorded responses with different wave shapes according to the position of the electrode. These variations included changes of polarity and waveform which were obviously related to a volume conductor effect. In order to facilitate experimental comparisons, several positions of the IAM electrode were successively tested until a criterion waveform was obtained. A criterion IAM waveform was obtained when the electrode was near the internal meatus showing a negative peak delayed by 0.25 msec compared with the AP. The positive peak was preceded by a slight positive voltage coincident with the onset of the negative peak. The delay in the negative peak probably corresponded to the conduction time to the electrode site at the IAM (Fig. 1).

Most of the characteristics of the centrally recorded neural response were similar to those of the cochlear first turn, i.e. the AP. Unlike the centrally recorded response, the first negative peak was the second being somewhat greater than the first and separated by a



The effect of KCl introduced at the internal auditory meatus upon the whole nerve AP response recorded from different basal turn electrodes. Curve *a* control waveform; curve *b* post KCl application; curve *a-b* difference between curve *a* and curve *b*. Curve *a-b* represents the effect of an active IAM upon the AP response recorded from the basal turn.

At some electrode locations the peaks appeared to correspond to  $N_1$  and  $N_2$  in the cochlear AP. At other positions of the electrode the wave shape could be quite different. The similarity between the AP (cochlear) and the IAM response is not true for all electrode locations. Owing to the latency this response could be attributed to first order neurons. However, in some records several late deflections were strong and probably originated in secondary or tertiary neurones. After introduction of the KCl solution at the IAM the response showed a rapid decrease in amplitude and a change in polarity and shape. Usually a small positive deflection remained which could be attributed to a passive transmission from the cochlea. At the same time the response (recorded by the electrodes in the cochlea) changed its shape and became monophasic as a positive deflection between  $N_1$  and  $N_2$  disappeared in such a way that the AP displayed a single wave of large amplitude. Fig. 1 illustrates the alterations in the waveforms from the cochlea and the IAM. Similar effects could be obtained with mechanical pressure produced with a probe in the region of the IAM. However, the effect of KCl was more progressive and easier to control. After rinsing with water a complete recovery of the initial response was observed.

In some instances the KCl solution apparently penetrated inside the cochlea since CM—as well as the cochlear AP—were reversibly depressed. When this cochlear depression occurred the experiment was rejected.

The exact correspondence between the disappearance of the potential recorded at the IAM and the change in the AP suggests that the positive deflection between  $N_1$  and  $N_2$  of the cochlear AP was generated at the IAM. Fig. 2 shows for a specific pair of waveforms the effect of KCl applied to the IAM. The waveform labelled *a* is the AP before application of KCl; that labelled *b* the waveform of the AP after application of KCl. The stimulus was at the same intensity for both waveforms. Waveform *b* follows the onset of *a* but reaches a larger amplitude just before *a* returns to the baseline and continues to its positive peak. Both waveforms then reach the baseline simultaneously: *a* from a positive voltage, *b* from its negative voltage. The difference between the two waveforms is shown as a third wave-

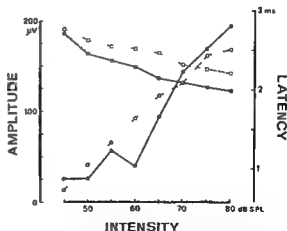


Fig. 3. Amplitude and latency of AP responses before and after application of pressure-block at the internal auditory meatus of the guinea pig. Stimulus: 8 kHz tone bursts; rise time is 1.0 ms. Pressure was produced by a small cotton ball placed at the IAM. The amplitude of the  $N_1$  response is greater for pressure block than for the control responses. The alteration in slope (55–60 dB) for control responses is eliminated for the responses during pressure block.



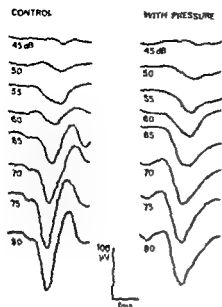


Fig. 4 Response waveforms from which measurements shown in Fig. 3 were taken

form (a-b). The difference in waveform represents the contribution of the intact IAM region, which was eliminated by the application of KCl.

With low frequency tone bursts or low pass clicks, the AP shows a smaller positive deflection but the effect of KCl on the IAM is similar and the  $N_1$  and  $N_2$  are fused together into a single wide negative deflection of large amplitude. The increase in amplitude of the AP is sometimes not very obvious because there is

apparently a diffusion of KCl towards the cochlea. In these cases, however, it is possible during the recovery to observe a transient state where the AP shows an amplitude markedly increased.

#### *Relation between the Shape of the AP and Its Intensity Function*

The increase in amplitude which follows the disappearance of the positive deflection suggests that some cancellation of the  $N_2$  peak has been removed when IAM is inactivated. This interpretation is consistent with the model of Teas et al. which describes the evolution of the neural elements according to their shape and synchrony. Because of change in shape of the neural elements to modify the resultant, i.e. the response waveform and its magnitude, we compared the variation in size of the responses as a function of intensity when recorded by the electrode located at the IAM and in the cochlea for (a) normal conditions and (b) after inactivation of IAM by KCl.

The intensity function of the  $N_1$  is well specified for the classical recording situation, i.e. round window in the cat or basal ferential electrodes in the guinea pig. In this series of experiments we measured peak to peak and also baseline to peak.

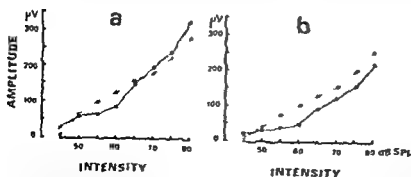


Fig. 5 Intensity functions measured as peak to-peak ( $N_1$  to positive notch) and baseline to-peak ( $N_1$  to negative peak) Stimulus: 8 kHz tone burst, rise time 1 ms. O: O post KCl application to internal auditory meatus. ●: ● pre KCl control responses. The two slopes of the

AP intensity function are apparent in the control conditions for the control responses. The slopes of the KCl responses do not show the characteristic change in the 45-60 dB range of intensity.

usually displays two branches, one with slope for intensities below 60 dB and one steeper slope at intensity levels above 60 dB.

At the junction between the two branches a dip is often observed. This shape has been considered by many authors as resulting from two groups of receptors activated separately by low and high intensities. Above 60 dB the curve is strongly non linear and a dip is observed, followed by a decrease in amplitude.

The inactivation of the central end of the nerve modifies the intensity function of the cochlear action potential.

Figure 3 shows plots of amplitudes and latencies of baseline to peak (negative) of the AP waveforms displayed in Fig. 4. Over the whole dynamic range the response observed while pressure was applied to the IAM was larger than the control response. The time when the peak occurred was also later for the pressure block condition. The change in latency for the pre-pressure block amplitude between 55 and 60 dB is absent in the response for the pressure-block condition. The pressure block response shows a nearly increasing function without the separation into the two segments, commonly observed in the intensity function.

Figure 5 shows plots of intensity function for responses to 8 kHz tone pips before and after KCl application to the IAM. The curves on the left are for peak to peak measures, on the right, for the baseline to peak measures.

Although the peak-to-peak measure is not in the observations (the post KCl response has little or no positive peak), the change in slope for the pre KCl response in the 10-20 dB region is lost in the post KCl growth response amplitude. The baseline to-peak measures show a clear and consistent increase in amplitude of the AP response following KCl application.

Relatively, with the changes in the amplitude-intensity function, the latency-intensity function is modified. In normal conditions, as

in classical, the curves representing the latency show two branches coincident with the two branches of intensity function. The first branch representing the latency at low intensities shows a steep slope, the second branch for higher intensities shows a lower slope. After KCl the latency shows a monotonic variation with intensity. These coincident changes in amplitude and latency argue that the amplitude is related to the time distribution or to the duration of the unit potentials.

## DISCUSSION AND CONCLUSIONS

The changes in the shape of the whole nerve AP have been observed by various authors who attempted to interrupt the auditory pathway at the level of the nerve trunk (Fisch & Ruben, 1962; Kupperman, 1972; Daigneault, 1974). The changes observed were usually interpreted as being the result of the removal of the influence of efferent fibres. The present results do not contradict this interpretation, but in our opinion it is more likely that the efferent action would change the overall amplitude rather than the wave shape. Comparison of the cochlear AP with the AP from the IAM after action of KCl strongly supports the interpretation that the IAM source participates in determining the waveform of cochlear AP.

The increase in size of the cochlear AP after the action of KCl could be related to the suppression of a tonic influence of efferent fibres. However the interruption of the olivo-cochlear pathway at other locations, in our experiments, did not produce a similar effect. It seems more likely that the principal action of KCl was to depolarize the nerve fibres at the IAM, thus blocking the conduction of nerve impulses, and sometimes also to modify the conductivity around the nerve trunk. While the change in conductivity could account for the increase in size the saline wash restored the control waveforms. Conductivity should be similar for both the solutions. The increase in size of the AP may result from the removal of the cancellation of negative deflection.

the addition of positive voltage, as can be deduced from the model of Teas et al

In subtracting the AP waveform recorded before KCl from the waveform obtained after suppression of the IAM response, the contribution of the IAM to the cochlear AP can be estimated. It appears as a positive deflection with a latency 0.3 ms longer than the cochlear AP. It is interesting to consider that after KCl the IAM electrode records also symmetrically a positive potential that we assume to be generated at the peripheral end of the meatus.

After the KCl action, the shape of AP becomes monophasic and  $N_2$  is fused with  $N_1$ . This alteration in waveform seems to result from the disappearance of the positive deflection which divides the response into two parts ( $N_1$  and  $N_2$ ). Several interpretations have been presented to explain the origin of  $N_2$ . It has been suggested that  $N_2$  represents the response of second order neurones. Tasaki (1954) asserted that it could be due to a second firing of auditory nerve fibres. The disappearance of  $N_2$  after KCl action suggests that it is more likely to be due to the removal of the positive deflection.

The intensity function of the whole nerve,  $P$ , has been studied by many authors. The curve which corresponds to it shows two branches which have been attributed to the stimulation of two different sets of receptors (Eggermont 1976). In some instances at the junction of the two curves there is a dip, the origin of which is difficult to explain. Recently it was proposed that the two branches were related to the shape of the tuning curve of single fibres (Evans 1975).

At low stimulus intensities tuning curves of auditory nerve fibres are very narrow and the AP response probably represents the activation of few nerve fibres from a narrow region along the cochlear partition. Thus the lower branch of the curve for the AP shows a small rise with intensity and the function has a low slope. At higher intensities the tuning curves are much wider. As intensity increases

the stimulus produces excitation along a large region of the cochlear partition and a large number of fibres produce impulses. The breadth of tuning curves for fibres with progressively higher characteristic frequency can account for the high intensity break in the intensity function. The rapid increase in amplitude, i.e. the steep slope, is due to the activation of fibres with high CFs (Kiang 1977). The present results show that the break in the curve disappears or is attenuated when the AP becomes monophasic after action of KCl. This alteration in the intensity function is clearly related to the response waveform. Thus the intensity function for the whole nerve response is also determined by the specific electro-anatomical features of the recording procedure imposed by the constraint resulting from the diphasic waveform.

The low intensity segment of the whole nerve intensity function represents the sum of pulses from a group of nerve fibres with characteristic frequencies within a narrow band around the stimulus frequency. Tones below 4 kHz or above, with rise times of 1 ms, are optimal stimuli for eliciting the response. At the lowest stimulus intensity there is a difference between the usual procedure (phasic response) and the response in the presence of an IAM block. As stimulus intensity increases the difference between pre- and post-block in the baseline-to-peak mean amplitude increases. The data suggest that the positive voltage erodes the initial negative component of neural elements at all but the lowest stimulus intensities, and the classical  $N_1$  response can underestimate the response magnitude by more than one half at the strongest stimulus values. Estimates of the number of neural elements on the basis of area encompassed in the classical  $N_1$  diphasic response are, of course, quite inadequate because the form is a complex resultant of the diphasic form of neural activity recorded in the cochlea.

This analysis of the whole nerve

the waveform shows that the response itself provides no basis for the inference that there are two populations of nerve fibres that respond selectively to the low and high intensity segments of the classical diphasic  $N_1$ -configuration. Under IAM block, when the waveform is monophasic, the whole-intensity function shows a uniform increase with saturation at high stimulus intensities.

The similarity in the form of the classical AP intensity function among species is due to the similarity of the electroanatomical inputs imposed by the configuration of the auditory nerve and internal auditory meatus. Differences among species will be in the relative contribution of excitation toward regions of high frequency with increases of intensity. These two factors may alter the specific features of response waveforms from human cochlea (electrocochleography) to cat, but not the general features.

## ZUSAMMENFASSUNG

Aktionspotential (AP) wurde mittels Intracochlearen Elektroden beim Meerschweinchen gemessen. Wie schon bekannt ergibt das durch Tonpulse ausgelöste AP unter normalen Bedingungen positive und negative Amplituden. Die Inaktivierung des Nervenendes im inneren Gehörgang (IAM) durch Aufbringen von einigen Tropfen Tetracain-Lösung oder durch mechanischen Druck bewirkt eine Umwandlung des AP in ein monophasisches Potential.

Die Intensitätsfunktion, welche unter normalen Bedingungen beobachtet wird, zeigt eine Zunahme der Amplitude mit der positiven Anteile der Antwort, welche

im IAM produziert werden erklärt. Die Existenz eines einheitlichen unitären APs wurde auch den monotonen Verlauf der Intensitätsfunktion erklären.

## REFERENCES

- Daigneault E A 1974 Source of the  $P_1$  component of the cochlea round window recording *Acta Otolaryngol* (Stockh) 77 405
- Davis II, Tasaka I & Goldstein R 1952 The peripheral origin of activity with reference to the ear *Cold Spring Harbor Symposia on Quantitative Biology* vol XVII
- Eggermont J J 1976 Electrocochleography in *Handbook of Sensory Physiology* vol 3 (ed W D Keidel & W D Neff) 625-706
- Evans E F 1975 The sharpening of cochlear frequency selectivity in the normal and abnormal cochlea *Audiology* 14 419
- Fisch U P & Ruben R J 1962 Electrical acoustical response to click stimulation after sectioning of the eighth nerve *Acta Otolaryngol* (Stockh) 54 537
- Kiang N Y S, Moxon E C & Kahn A R 1977 The relationship of gross potentials recorded from the cochlea to single unit activity in the auditory nerve. In *Proceedings of the Symposium on Electrocochleography* (R Ruben, C Elberling and G Salomon eds)
- Kupperman R 1972 Cochlear adaptation central influences *Acta Otolaryngol* (Stockh) 73 130
- Legoux J P & Pierson A 1974 Investigations on the sources of whole nerve action potentials recorded from various places in the guinea pig cochlea *J Acoust Soc Am* 56 1222
- Tasaka I 1954 Nerve impulses in individual auditory nerve fibers of guinea pig *J Neurophysiol* 17 97
- Teas D C, Eldredge D H & Davis II 1962 Cochlear responses to acoustic transients: an interpretation of whole nerve action potentials *J Acoust Soc Am* 34 1438

J P Legoux MD  
Lab de Neurophysiologie  
Collège de France  
11 place Marcelin Berthelot  
75231 Paris Cedex 05  
France

## CLINICAL FINDINGS AND DIAGNOSTIC PROBLEMS IN SENSORINEURAL LOW FREQUENCY HEARING LOSS

A. Parving and K. Bak Pedersen

*From the Audology Clinic ENT Department Gentofte University Hospital Hellerup Denmark*

(Received April 20 1977)

**Abstract** The otological and audiological findings in 39 patients with sensorineural low frequency hearing loss are reported. This type of perceptive hearing loss is difficult to distinguish from true conductive hearing losses due to the air conduction audiogram shape and the invalidity of bone conduction determinations showing a false air-bone gap. This may lead to surgical treatment of a perceptive hearing loss as reported in the four case histories. By various audiological tests contradictory information may be obtained. In our material Ring's test and absent acoustic reflexes indicated a conductive disorder in 25% of the ears. The final differentiation may require cochleography. The hearing loss may be diagnosed as Meniere's disease. In our material only 17% complained of tinnitus and no patients had vertigo. Consequently we find sensorineural low frequency hearing loss to differ from Meniere's disease. Our material comprises different etiological types of perceptive low frequency hearing loss. One type was inherited as an autosomal dominant trait, another type due to cochlear malformation probably also inherited, and a third group showing diverse audiological results. When the diagnosis is established the patients may be treated successfully by specially constructed hearing aids.

Hearing loss may be classified as mainly conductive or sensorineural. Clinically, it may be difficult to distinguish between these components due to misleading bone conduction determinations (Lierle & Reger 1946). It is of great clinical importance to differentiate between these two main types, as a conductive hearing loss may be treated surgically. Surgical treatment of a perceptive hearing loss may fail to improve the hearing or even worse result in further impairment of the hearing ability. Also, from a psychological point of

view the patient suffers when the hope of improved hearing is not fulfilled, and it may become difficult to motivate such patients to wear a hearing aid, postoperatively.

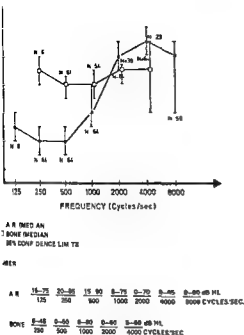
Conductive hearing loss is attributable to a variety of pathological conditions in the ear canal or in the middle ear. Congenital malformation, post-traumatic disorder, acute chronic infection or sequelae of infection

maieus syndrome and otosclerosis (Wu 1966, Elbrond, 1970).

Conductive hearing loss is normally characterized by an air conduction audiogram shape which shows a more pronounced hearing impairment in the low frequency area and a decreasing loss towards the treble. This audiometric curve has been named the stiff tilt (Carhart, 1962). The bone conduction thresholds are within normal range but in some disorders the bone conduction thresholds are measured too low, simulating a perceptive hearing impairment (McConnell, Carhart, 1952, Tonndorf, 1971).

Sensorineural hearing loss is caused by pathology in the cochlea, the cochlear nucleus or central auditory pathways. It is characterized by progression, heredity and may have associated abnormality with other systems (Königsmark, 1969, 1971a, 1971b).

The common type of sensorineural hearing



1 The median air and bone conduction thresholds ears examined

is characterized by hearing impairment, pronounced in the high frequency area, a sloping audiogram. A special type of sensorineural hearing loss is seen in the low frequency area, defined as a hearing loss most pronounced for the frequency range below 2 kHz and exceeding 20 dB or more. The hearing may be better for 125 Hz than for other frequencies (Königsmark et al., 1971; van der Aalst & Plomp, 1960; Lundborg, 1955; Sweeney, 1975).

To elucidate the diagnostic problems, we will report the otological and audiological findings in the patients with this type of hearing loss and evaluate the diagnostic procedures available.

## METHOD AND MATERIAL

In this study, the patients were selected consecutively according to the otoscopy and the audiogram shape showing hearing loss most pronounced for the frequency range below 2 kHz and exceeding 20 dB or

more. The air conduction thresholds were obtained with Telephonics/TDH 39 earphones with MX 41/AR cushions, meeting the specifications given in recommendation IEC/177-1965 and calibrated according to recommendation ISO/R 398-1964/Add 1-1970. The bone conduction thresholds were obtained with Oticon A-43 bone conductors applied to the mastoid process, meeting the specifications given in recommendation IEC/177-1965/App A and calibrated on a Brüel & Kjær artificial mastoid type 4930 using "acceleration"—calibration values from Flottorp (1972) (see also Dahm, 1973).

Diagnostically, the patients fell in two groups. One group showed cohesive test results, i.e. normal otoscopy, positive Bing, normal middle ear pressure, and stapedial reflexes present. In contrast, the second group was difficult to evaluate because of contradictory test results, i.e. normal otoscopy, normal middle ear pressure, negative Bing, and no stapedial reflexes. In the latter group further audiological tests were done: Fowler's ABLB, when possible, caloric testing, brief tone audiometry, X-ray tomography, exploration of the middle ear, and, finally, cochleography by means of an ear canal electrode (Salomon & Elberling, 1971).

During a five-year period 39 patients, 11 males and 21 females, were found according to the aforementioned criteria. The age ranged from 6 to 75 years, average 42.6 years. Twenty-five patients had bilateral symmetrical hearing loss, 14 patients had monaural affection. A total of 64 ears were affected. Besides the procedures used for selection and diagnosis, speech reception threshold (SRT) and discrimination loss (DL) with a monosyllable phonetically non-balanced word list were determined in all patients (Danielsen et al., 1971). In 58 ears Bing's test was done. Impedance measurements were performed with a Madsen Electronic acoustic impedance bridge model ZO 70. The acoustic reflex thresholds were determined by contralateral stimulation in 61 ears. In 3 patients it was pos-

Table 1 Results of the speech reception thresholds and discrimination loss for all ears examined

	SRT	DL
Median	20	0
95% confidence	15-25	0-0
Range	0-45	0-25

sible to make Fowler's ABLB test and in 5 patients caloric testing and tone-decay test were performed. Bilateral brief tone audiometry was performed in 4 patients and 8 patients were examined by X-ray tomography of the inner ear. Three patients were subjected to cochleography and 4 patients had their middle ear explored, as the hearing loss was at first considered to be of a conductive type. Three patients had tubes inserted because of chronic secretory otitis media.

## RESULTS

### Anamnesis

Thirteen patients (33%) had a family history of hearing impairment, while 20 patients (51%) were without known predisposition. The hearing losses had a duration of as little as 2 months to as long as 15 years (average about 12-14 years). Ten patients (25%) had noticed worsening of the hearing loss. Seven patients (17%) complained of tinnitus, while no patients had vertigo.

### Objective findings

By otoscopy 3 ears (5%) were classified as simple glue ears. After adequate treatment, the otoscopies were normal, but the audiograms continued to show hearing losses in the low frequency area. The median air- and bone conduction thresholds are seen in Fig. 1 showing an "air-bone gap" of the low frequency area. Furthermore, the hearing is normal at 2 kHz and 4 kHz. Table I shows the results of the SRT and DL values. Table II shows the

percentage of ears where the results of various audiological tests indicated conductive or perceptive hearing loss. In 52 ears (81%) middle ear pressure was within normal range. By contralateral stimulation the middle ear reflex could be elicited in 44 ears (68%) in 11 ears at a threshold indicating recruitment. Three ears showed recruitment with Fowler's ABLB test which indicated cochlear damage. In 2 siblings the X-ray tomography showed inner ear defects of the Mondini type; the others were normal. The cochleography performed in 3 patients revealed, in all 3, signs of hair cell degeneration but no signs of impairment in the transmission system.

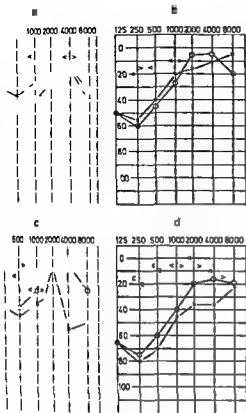
The patients who had their middle ear explored by operation due to an erroneous diagnosis will receive more detailed comments.

### Patient 1

A girl, born 1957, first seen at the Otology Department for tonsillectomy, 1969. At admission an audiogram was made which showed bilateral air conduction thresholds at 40-50 dB HL in the low frequency area and normal bone conduction thresholds (Fig. 2a). The patient underwent myringotomy, where middle ear effusion was found, removed and treated with tubes. No hearing improvement occurred until the tubes were removed. One year later repeated audiological examination showed unchanged hearing thresholds. Bing's test was negative. The middle ear pressure was normal. A small stapedial reflex could be elicited by contralateral stimulation with a Barany's

Table II Percentage of ears pointing towards conductive and perceptive hearing loss by otoscopy, Bing's test, and acoustic stapedial reflex

	Otoscopy	Bing's test	Acoustic reflex
Conductive	5%	25%	26%
Perceptive	95%	65%	68%



2 Audiograms of four patients (cases 1-4) with low frequency sensorineural hearing loss who had their middle ear explored due to an erroneous preoperative diagnosis

The X ray tomography was normal and was concluded that a probably slight ossicular fixation was present. The right middle ear was explored and the stapes was palpated tightly fixed, but no otosclerotic focus was found. The incus and malleus were mobile. The oval footplate was mobilized, but no improvement occurred.

The patient was last seen in May 1975. The hearing was normal. The audiogram was completely unchanged. Bing's test was still negative, middle ear pressure -250/-200 mm Hg, small acoustic reflexes could be elicited, and brief tone audiometry gave a normal conduction within the air conduction range. Cochleography performed in 1971 showed no abnormality in the hair cells. The patient was successfully treated with hearing

aids. Conclusion: The patient has a sensorineural hearing loss in the low frequency area.

### Patient 2

Girl, born 1955, first seen at this department in 1971. She complained of hearing loss lasting for 7-8 years. Otoscopy was normal and the audiogram confirmed a bilateral hearing loss in the low frequency area (Fig. 2b). Bing's test was negative, middle ear pressure normal, bilateral stapedial reflexes were present with no signs of recruitment and no intensity variation was found by Gelle's test. It was concluded that the patient had a conductive hearing loss due to some loose connection in the ossicular chain and her right middle ear was explored. The ossicles were found to be normal, but no definite hydrodynamic effect was seen in the round window upon movement of the stapes. This finding was inexplicable. The postoperative audiogram showed unchanged hearing. The patient was last seen in 1972 and her hearing impairment was unchanged. Treatment with hearing aids was unsuccessful. Conclusion: The patient has a sensorineural hearing loss in the low frequency area.

### Patient 3

Girl, born 1957 with hearing impairment of uncertain type from 10 years of age. In 1971, when first seen at this department, the otological examination was normal and the audiogram was as shown in Fig. 2c. Bing's test was negative, middle ear pressure normal, no stapedial reflexes could be elicited, but intensity variation was seen by Gelle's test. X ray tomography was normal. It was concluded that the patient had malformation in the middle ear and this was explored. The ossicles were intact, but by palpation the surgeon got an impression of a slight fixation of the stapedial footplate. It was mobile, but gave no movement of the round window membrane, thus leading to stapedectomy *ad modum* Shea. No hearing improvement occurred and when last seen in 1974, the audiogram showed no change since the first



1971 The patient is successfully treated with hearing aids. Conclusion The patient has a sensorineural hearing loss most pronounced in the low frequency area.

#### Patient 4

A boy, born 1965, admitted to the department in 1972 because of otosalingitis. He had complained of hearing impairment for half a year. The audiological examination revealed bilateral hearing loss for lower frequencies (Fig 2d). Middle ear pressure was normal and no stapedial reflexes could be elicited. Bing's test was negative. Otomicroscopy showed signs of fixed malleus syndrome. His left middle ear was explored and some fixation of the ossicular chain was found. The fixation was loosened, but only 10 dB HL improvement was obtained. Due to the unsuccessful treatment, the patient was again admitted to the hospital for operation of the right ear. On palpation the same impression of ossicular fixation in this ear was present. The incus was removed and reinserted on the stapes with crus longum pointing downwards and crus breve under the malleus. Thereafter, reflex was seen in the round window by movement of the stapes. After the operation a hearing improvement of 10 dB HL was seen at all frequencies, but still a pronounced hearing loss was present in the lower frequency area. When last seen in Oct. 1976 the audiogram was unchanged. The patient is successfully treated with hearing aids. Conclusion The patient has a sensorineural hearing loss for lower frequencies combined with a slight ossicular fixation.

#### DISCUSSION

The difficulties in distinguishing between true conductive hearing losses and low tone perceptive hearing losses can be ascribed to 1) both may have the same shape of the air conduction audiogram, and 2) misleading bone conduction measurements showing a false "air-bone gap", and 3) in the low frequency

sensorineural hearing loss special problems arise from the contradictory information obtained by different audiological tests.

As earlier mentioned, hearing loss for frequencies is mostly seen when some fixation is present in the sound transmission system showing a stiffness tilt. But reported, the hearing loss may be due to a kind of abnormality in the hair cells of the apical part of the cochlea (Schuknecht & 1952, Vanderbilt University Hereditary Deafness Study Group, 1968). Some authors regard the patients as being completely deaf in the low frequency area (Gravendeel & 1960). They believe that the measured losses in this frequency area are answer to harmonics, of which the higher frequencies consist, which may have sufficient intensity to be heard.

As mentioned in the introduction, if conduction thresholds in some cases of hearing losses are measured too low, indicating a perceptive hearing loss and in sensorineural hearing loss it is vice versa. The accuracy of bone conduction determinations due to technical and biological phenomena. In connection with perceptive hearing loss is relevant to mention just a few of the technical reasons for the invalidity of bone conduction. The occlusion effect (Goldstein & 1965, Tonndorf et al., 1966), the jaw effect (Schuchman & Burgi, 1971), and the round window release phenomenon (Tonndorf, 1962). Furthermore, it has been shown that below 1000 Hz, harmonic distortion products only 20 dB below the levels of the fundamentals (Khanna & 1976). Furthermore, an absolute systematic error of bone conduction determination of 15 dB may be present (Carhart & 1949). To avoid traditional bone conduction determinations, alternative methods are used, such as the SAL-test or brief tone audiometry (Jerger & Tillman, 1960, Pedersen & Salomon, 1977).

From a communicative point of view

will manage very well because the ability is preserved in the high frequency area. Due to this preservation, simple tests will not reveal this type of hearing loss since whispered speech is heard better than voiced speech (Ross & Matkin, 1967). When the patients for different reasons are referred for examination, the contradiction between information obtained by audiological and otological findings is obvious from the fact that Bing's test of the impedance measurements pointed out conductive hearing loss in 25% of the cases.

The contradiction is also apparent in the 4 referred case histories, where the otological and audiological findings are in contradiction. In our opinion the diagnosis of sensorineural hearing loss in the low frequency area is often very difficult and must depend on the results of cochleography (Elberling & Salomon, 1976).

The hearing loss is most pronounced in the low frequency area. It may be diagnosed as Meniere's disease (Enander & Stahle, 1967).

It is also progressive and may occur at any age (Sørensen, 1959; Harrison, 1962; Parving, 1967).

None of our patients had vertigo, but 50% had progression of hearing loss, and only 2 of them complained of tinnitus.

Other authors have also found sensorineural hearing loss in the low frequency area different from the well known low frequency hearing loss in Meniere's disease (Jama et al., 1967).

(1965) and Carhart (1962) believe that the low frequency sensorineural hearing loss may be indicative of labyrinthine otosclerosis. The X-ray tomography performed in our patients was normal except in 2 cases showing Mondini defect, but in the absence of evidence it is not possible to exclude morphologic alteration of the organ of Corti as a possible cause of the hearing loss.

The material of low frequency hearing loss consists of different etiological types. One important type is well known as an autosomal recessively inherited trait which means that the

possession of a single abnormal gene is sufficient for full manifestation of the trait (Nance & Sweeney, 1975; Nance et al., 1970; Nance, 1971). Another type due to cochlear malformation according to the Mondini type is probably also inherited, but our results permit no conclusion to be drawn on the mode of transmission. A third group showed a mixture of audiological findings, probably consisting of several different types as no certain entity could be outlined.

When the diagnosis is fully established the patient should be treated with hearing aids, if the degree of hearing loss so indicates. This treatment is often not successful, as the hearing ability is normal in the high frequency area giving a good discrimination ability (Aniansson, 1972). This good discrimination ability may be spoiled by distortion, acoustic feedback, and by other disadvantages of wearing a hearing aid. Moreover, most available hearing aids have a frequency characteristic and amplification which is not suited to this type of hearing loss, although a few hearing aids with only low frequency amplification have been constructed. As abnormal behaviour due to secondary psychological problems has been reported in this type of hearing impairment, hearing aids should always be tried (Ross & Matkin, 1967).

## ZUSAMMENFASSUNG

Die otologischen und audologischen Befunde bei 39 Patienten mit niederfrequent sensorischer Schwerhörigkeit wurden referiert. Diese sensorische Schwerhörigkeit ist schwierig zu diagnostizieren, da die Audiogrammform ähnlich konduktiver Schwerhörigkeit ist und weil die Bestimmungen der Knochenleitung unsicher sind. In niederfrequent sensorischer Schwerhörigkeit sieht man ein air-bone gap. Dieses kann auf Grundlage eines fehlartigen Befunds zur Operation führen. Die Schwerhörigkeit in Menierescher Krankheit ist ähnlich der niederfrequenten sensorischen Schwerhörigkeit, sondern kein Schwindel ist hier anwesend. Das Material zeigt, daß verschiedene Typen von niederfrequent sensorischer Schwerhörigkeit unterschieden werden können: 1) Der dominant erbliche Typ, 2) ein Typ mit Malformation in dem inneren Ohr, vielleicht auch erblich, und 3) ein Typ mit verschiedenen und kontroversiellen audio-

logischen Funden. Wenn die Befunde feststehen, sollen die Patienten mit speziell konstruierten Hörapparaten versuchsweise behandelt werden.

## REFERENCES

- Aniansson, G. 1972 Methods for assessing high frequency hearing loss in everyday listening situations *Acta Otolaryngol* (Stockh), Suppl. 302
- Carhart, R. 1962 Effect of stapes fixation on bone-conduction response. In *Otosclerosis* (ed. H. F. Schuknecht). Little Brown, Boston.
- Carhart, R. & Hayes, C. 1949 Clinical reliability of bone conduction audiometry *Laryngoscope* 59, 1084.
- Dahm, M. 1973 Calibration problems in bone vibration with reference to IECR 373 and ANSI S3.13-1972 *Bruel & Kjaer Technical Review* 2, 31.
- Danielsen, H., Elberling, C. & Salomon, G. 1971 Experimental monosyllable discrimination test for evaluation of hearing aids *Nord Audiol* 46, 45.
- Elberling, C. & Salomon, G. 1976 Action potentials from pathological ears compared to potentials generated by a computer model. In *Proc Symp Electrocochleography* (ed. R. J. Ruben, C. Elberling & G. Salomon). University Park Press, New York, 1974.
- Elbrønd, O. 1970 Defects of the auditory ossicles in ears with intact tympanic membrane *Acta Otolaryngol* (Stockh), Suppl. 264.
- Enander, A. & Stahle, J. 1967 Hearing in Menière's disease *Acta Otolaryngol* (Stockh) 64, 343.
- Flottorp, G. 1972 Bone conduction threshold data. Institute of Audiology, University of Oslo. Unpublished data.
- Carhart, R. & Hayes, C. 1965 The occlusion effect in bone-conduction hearing *J Speech Hear Res* 8, 137.
- Carhart, R. & Jerger, J. 1966 External conducted hypoacusis and the fixed malleus syndrome *Acta Otolaryngol* (Stockh), Suppl. 217.
- Gravendeel, D. W. & Plomp, R. 1960 Perceptive bass deafness *Acta Otolaryngol* (Stockh) 51, 548.
- Harrison, M. S. 1962 Vertigo in childhood *J Laryng Otol* 76, 601.
- Iinuma, T., Shitara, T., Hoshino, T. & Kinkae, I. 1967 Sensorineural hearing loss for low tones *Arch Otolaryngol* 86, 110.
- Jerger, J. F. & Tillman, T. A. 1960 A new method for the clinical determination of sensorineural acuity level (SAL) *Arch Otolaryngol* 71, 948.
- Khanna, M., Tonndorf, J. & Queller, J. E. 1976 Mechanical parameters of hearing by bone conduction *J Acoust Soc Amer* 60, 1.
- Königsmark, B. W. 1969 Hereditary deafness in man *N Engl J Med* 281, 774 & 827.
- 1971a Hereditary deafness syndrome with onset in adult life *Audiology* 10, 257.
- 1971b Hereditary congenital severe deafness syndromes *Ann Otol* 80, 270.
- Königsmark, B. W., Mengel, M. & Berlin, C. L. 1971 Familial low frequency hearing loss *Laryngoscope* 81, 758.
- Lierle, D. & Reger, S. 1946 Correlations between air and air conduction acuity measurements in different frequency ranges in different types of hearing impairments *Laryngoscope* 56, 187.
- Lundborg, T. 1955 Nerve deafness for low tones *Acta Otolaryngol* (Stockh) 45, 215.
- McConnell, F. & Carhart, R. 1952 Influence of stapes surgery on bone conduction measurement *Laryngoscope* 62, 1267.
- Nance, W. E. 1971 Genetic counseling for the hearing impaired *Audiology* 10, 222.
- Nance, W. E. & Sweeney, A. 1975 Genetic factors in deafness of early life *Otolaryngol Clin Amer* 18, 1.
- Nance, W. E., Sweeney, A., McLeod, A. C. & Comp, M. C. 1970 Hereditary deafness: A presentation of some recognized types, modes of inheritance and aids in counseling *South Med Bull* 58, 4.
- Parving, A. 1976 Menière's disease in childhood *Laryng Otol* 50, 817.
- Pedersen, C. H. & Salomon, G. Conductive hearing loss evaluated by brief tone audiometry *Acta Otolaryngol*. In press.
- Ross, M. & Matkin, M. D. 1967 The rising acoustic metric configuration *J Speech Hear Dis* 32, 377.
- Salomon, G. & Elberling, C. 1971 Cochlear nerve action potentials recorded from the ear canal in man *Acta Otolaryngol* (Stockh) 71, 319.
- Shambaugh, G. E. Jr. 1965 Clinical diagnosis of otosclerosis (labyrinthine) otosclerosis *Laryngoscope* 75, 1558.
- Schuchman, G. & Burgin, E. J. 1971 The effect of position on hearing by bone conduction *J Aud Res* 124, 124.
- Schuknecht, H. F. & Neff, W. D. 1952 Hearing loss after apical lesions in the cochlea *Acta Otolaryngol* (Stockh) 42, 263.
- Sorensen, H. 1959 Menière's syndrome in a seven old girl *J Laryng Otol* 73, 346.
- Tonndorf, F. 1971 Animal experiments on bone conduction. Clinical conclusion. In *Hearing Measurement* (ed. T. Ventry, J. Chaiklin & H. Dixon). McGraw-Hill, New York.
- Tonndorf, F., Greenfield, E. C. & Kaufmann, R. S. 1971 The occlusion of the external ear canal its effect upon bone conduction in cats *Acta Otolaryngol* (Stockh), Suppl. 213, 80.
- Tonndorf, F. & Tabor, J. W. 1962 Closure of the ear canal windows *Ann Otol* 71, 5.
- Vanderbilt University Hereditary Deafness Study. 1968 Dominantly inherited low frequency hearing loss *Arch Otolaryngol* 88, 1.

A. Parving, M.D.  
Audiology Clinic  
Gentofte University Hospital  
DK-2900 Hellerup  
Denmark

SOME EXPERIMENTS ON TEMPORARY THRESHOLD SHIFTS  
PRODUCED BY SHORT TONES

E. Pirodda and A. Rinaldi Ceroni

*From the Department of Otolaryngology, University Hospital, Bologna, Italy*

(Received November 16, 1976)

tract TTSs produced by short (500-1000 msec) pure  
quency (2000-3000-4000 cps) tones on normal  
ng subjects show, under comparable conditions, the  
ne essential characteristics as TTSs which follow  
per stimulations. In particular, they show a tendency  
a displacement of their maximal values, as soon as the  
relating tone is presented at adequately high (100 dB  
L) intensity levels, towards a frequency about a half  
ave higher than that of the stimulating tone. In our ex-  
periments, however, this tendency was more clearly and  
stantly demonstrable following stimulations with 3000  
s tones. Significant individual variations in quantita-  
e aspects of the TTSs have been observed. It is sug-  
gested that the use of short tones may be easier and more  
licable in practice in investigations concerning the  
e effects of acoustical stimulation and possibly also in  
cedures aimed at obtaining a better understanding of  
e significance of individual variations in the behaviour  
the receptor organ upon exposure to high intensity  
stimulations.

studies concerning some after effects (tem-  
orary threshold shift) which can still be de-  
onstrated immediately after the cessation of  
stimulation by short (500-1000 msec) pure  
ones presented at increasing intensity levels  
ave been periodically carried out by many in-  
estigators, as a way of obtaining information  
bout the functional behaviour of the receptor  
rgan.

A survey of the most significant results by  
arly investigators, along with a personal con-  
tribution, was published in 1953 by O. Bent-  
sen on the occasion of his doctoral disserta-  
ion.

The characteristic pattern shown by the

threshold shift as a function of the intensity  
level of the stimulating tone and of the fre-  
quency of the test tone, is widely known. In  
essence, at low and moderate intensity levels,  
the maximal threshold elevation occurs for a  
test tone of the same frequency as that of the  
stimulating tone. Higher frequencies show a  
threshold shift gradually decreasing in magni-  
tude as the frequency of the test tone in-  
creases, the extension of the affected area de-  
pending on the intensity level of the stimulat-  
ing tone. Lower tones show practically no sig-  
nificant threshold shift.

It has been observed, however (among  
others by Zwislocki and by one of the present  
authors) that as soon as the intensity of the  
stimulating tone reaches high SP levels (90-  
100 dB) such a characteristic and monotonous  
pattern shows a remarkable tendency to signif-  
icant changes.

The maximal threshold shift is no longer  
clearly and typically confined to the frequency  
of the stimulating tone, but shows a more or  
less pronounced tendency to reach a second  
maximum at a frequency about one half oc-  
tave higher. Fairly frequently the maximal  
threshold shift is clearly displaced to such a  
frequency.

The behaviour of the threshold shift pro-  
duced by low and moderate intensity tones  
seems to find a reasonable and easy interpreta-  
tion as a state of residual excitation (residual

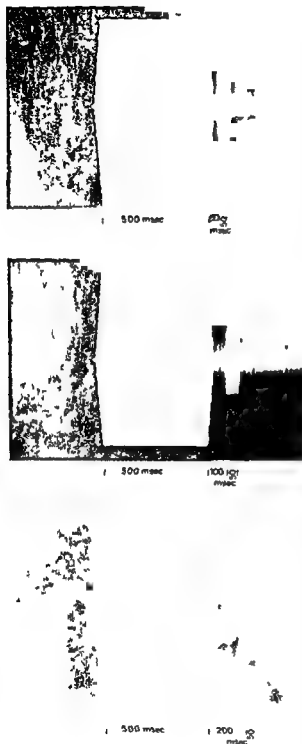


Fig. 1 Sequences of tones as used in the different experiments

masking adaptation or related phenomena). It seems to represent a physiological process to reproduce in some way the pattern of the mechanical process of excitation and to be

correlated with the arrangement of the 'endings' distribution along the cochlear duct.

The onset of a second maximum, the displacement of the maximal threshold shift to a frequency one half octave or more above that of the stimulating tone can be explained on in terms of a new, different situation. The previous inference is that such a phenomenon may be linked to some sort of impairment of a certain number of functional units to be considered as a consequence, although temporary, of the mechanical stress to which they have been submitted (Zwislocki & Pirodda 1952, Bentzen 1953 etc). The analogy with quite similar effects produced by longer stimulations obviously supports such an interpretation. Zwislocki was able to give a mathematical analysis of the mechanical factors involved, and thus to find a theoretical explanation for this displacement of the maximal threshold shift, showing that there is a mechanical magnitude, in the process of excitation (the apparent acoustic power per unit length of the cochlear duct) the local maximum of which is shifted, with respect to the place of maximum vibration, towards the base of the cochlea.

Even for short tones, furthermore, the duration of the recovery time of threshold shift produced by high intensity levels is more than significantly longer than the few milliseconds needed for the restoration of the presynaptic threshold when stimulations of low to moderate intensity levels are involved.

As a practical consequence, the TTS produced by stimulations carried out at high intensity levels may still be clearly demonstrable when the pure residual excitation produced by low and moderate intensity levels has rapidly disappeared.

Finally, even when short tones are used, the magnitude and the duration of the TTS produced by high intensity stimulations show some relationship to the duration of the stimulus, which does not happen for a short duration after effects elicited by low and moderate intensity levels.

In short, the qualitative similarity between

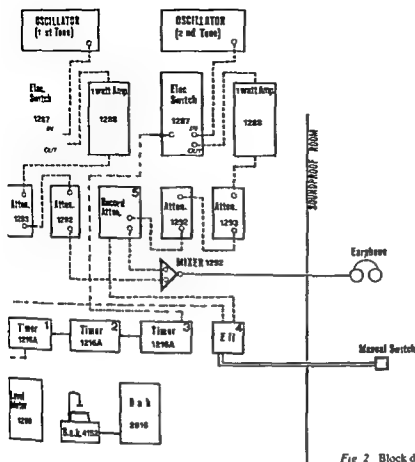


Fig 2 Block diagram of the equipment

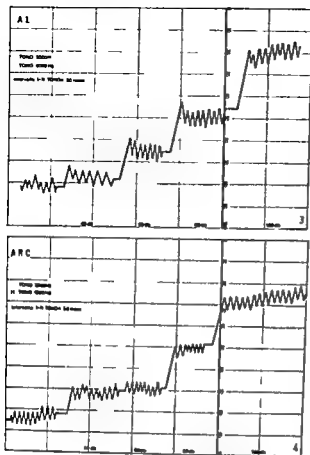
• effects produced by short stimulations  
 • effects produced by long stimulations is  
 mplete

It is well known that much work has been  
 ne on the generally accepted assumption  
 it some kind of correlation exists between  
 TS and PTS produced by exposure to noise  
 Practical application of the general principle  
 hich can hardly be overlooked) to the in-  
 vidual situation and to the prediction of in-  
 vidual reaction to exposure to noise is, how-  
 er, highly controversial (Ward et al., 1959,  
 rns & Robinson, 1970, among many others)  
 d subject to many uncertainty factors  
 Consequently, we felt that further experi-  
 ents on the behaviour of after-effects follow-  
 g stimulations by short tones would be justi-  
 fied, with the aim of collecting additional data

and in the hope that some day, in some way,  
 they may be useful in working out a reliable  
 clinical application of such procedures for  
 the evaluation of individual susceptibility to  
 acoustic overloading. The use of short tones  
 should provide some advantages: a shortening  
 of the test time, and perhaps a safer and more  
 easily accepted experimental condition than  
 those corresponding to procedures involving  
 longer stimulations.

#### SUBJECTS EQUIPMENT, EXPERIMENTAL PROCEDURES

Since one of the primary purposes of the pre-  
 sent investigation was to collect highly reliable  
 data (many discrepancies are to be found in



Figs 3-4 Some samples of threshold tracings obtained from tested subjects. Note the tendency to a progressive increment of the TS at the 100 dB intensity level

the findings of previous and especially early investigators, which seem in most cases to be attributable to technical factors) experiments were carried out on a small group of 20 selected, young (under 30 years) healthy, normally hearing and well trained subjects.

Only pure frequency tones of 2000, 3000 and 4000 cps were used as stimulating tones. Their duration was either of 500 or of 1000 msec during different experimental sessions. The rise-decay time was kept within values (10-25 msec) from which no significant interference with experimental results was to be expected. The intensity levels of the stimulating tone used in the present experiments were 40, 60, 80 and 100 dB (SPL).

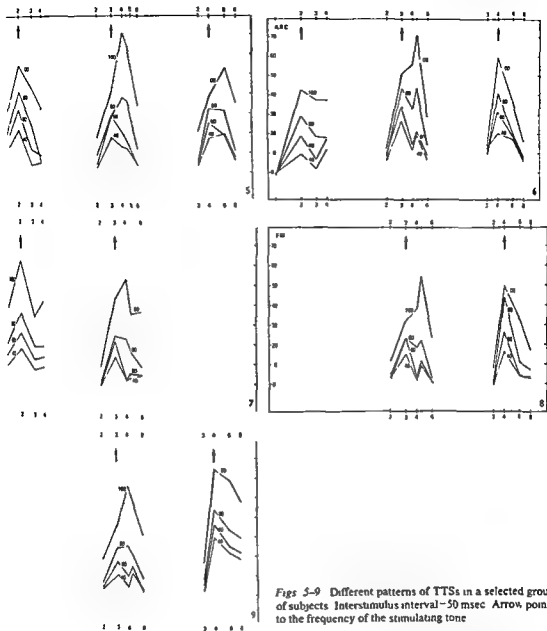
The threshold shift was determined by a test

tone of 50 msec duration (rise-decay 1 msec) (Fig. 1). In order to achieve a primary orientation, and to try to reduce as far as possible the duration of the test procedure, a limited number of frequencies was tested, the attention being focused on the frequency one half octave higher than that of the stimulating tone. On the basis of previously established facts, the interval between the stimulating and the test tone was kept constant (500 msec peak-to-peak) in the majority of the experiments; on some subjects, however, intervals of 100 and 200 msec were used on assumption, as mentioned before, that no loading phenomena should have shown longer persistence, thus allowing a better easier demonstration of their presence and a more rapidly disappearing short duration threshold shift due to residual adaptation. In addition, a longer interval between the tones should make easier the determination of the threshold shift, particularly for untrained subjects.

The block diagram (Fig. 2) shows the essential characteristics of the equipment which has been used. Checking of functions of different units and frequency and amplitude calibrations were repeated accurately before and after every experiment session.

In order to achieve the best accuracy in measurement of the threshold shift, the threshold tracking procedure has been used: the mean value calculated for each intensity level taken as the final value. A Grason-Stamper recording attenuator model E 3262 had been modified for our experimental requirements in order to eliminate the on and off-effect generated by the relays which produced distortion in the stimulating tone.

Such a procedure implied of course repeated presentation of the sequence of two signals at any intensity level of the stimulating tone. Each single train was triggered by the tested subject himself by operating a key, at any desired moment when he felt prepared to concentrate on the task of making a very careful and attentive judgement. The



Figs 5-9 Different patterns of TTSs in a selected group of subjects. Interstimulus interval—50 msec. Arrow, points to the frequency of the stimulating tone

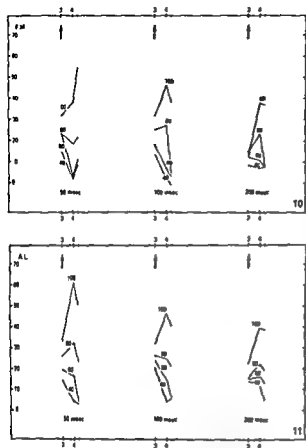
placement of the key elicited an increment the downward displacement a decrement by 1 dB of the intensity of the test tone in corresponding sequence of stimuli. By this procedure, the tested subject was required to make a decision every time he triggered a train of signals, whether he heard the tone or not, and decide, consequently whether to increase or decrease its intensity in successive sequence.

Some examples of traces obtained by this procedure from different subjects are given in Figs 3 and 4.

## RESULTS

Experiments, even on young trained subjects, proved to be more time-consuming and their results somehow less predictable than ex-





Figs 10-15 Comparison among TSs as measured at 50 100 200 msec after the cessation of the stimulating tone. The displacement of the maximum threshold elevation to a frequency a half octave higher is always demonstrable.

ted on the basis of previous research; therefore, only preliminary results can be given at the present.

Some essential findings seem to emerge with unquestionable evidence from the experiments which have been performed up to the present time, namely:

(1) In contrast to some previously accepted views, in agreement with others, the shift of the maximum threshold elevation to higher frequencies, and especially to a frequency about one half octave higher than that of the stimulating tone, does not occur as a constant phenomenon and in any case does not show the same pattern for any frequency of the stimulating tone, as shown in the graphs which illustrate individual findings from some selected subjects (Figs 5-9).

Even in our experiments the displacement of the maximal threshold shift could be observed almost constantly, and sometimes with impressive quantitative characteristics when the frequency of the stimulation tone was 3000 c.p.s. (Figs 5-9) which is in agreement with the results of experiments carried out by employing longer stimulations. Once again, even when after effects following short tones are concerned, the area of the cochlear receptor corresponding to the frequencies of 4000-4500 c.p.s. shows a critical sensitivity to mechanical damage.

Such a behaviour must be submitted to further investigation especially, in our opinion, as regards its variability in one and the same individual. It should represent an useful indication in view of a possible application of these procedures to clinical purposes.

(2) The magnitude of the observed threshold shifts for frequencies higher than that of the stimulating tone has shown even in our experiments, a very wide individual variability (Figs 5-9).

(3) The repetition of the stimulation even at relatively long time intervals when the stimulating tone was presented at the highest intensity level (100 dB), occasionally produced some kind of cumulative effect: a tendency to a progressive increment of the threshold shift as demonstrated by the ascending pattern of the corresponding trace (Figs 3-4) from individual cases.

After effects following stimulations by short tones, in other words, behave, from a qualitative point of view, in exactly the same way as temporary threshold shifts produced by longer stimulations.

In the present situation therefore they should receive greater consideration when planning further research on the intriguing and complex problem of the TTS/PTS relationship. The study of their behaviour could provide a means of obtaining highly reliable information by a relatively simple and time-sparing procedure, easy to perform under routine clinical conditions.

## ZUSAMMENFASSUNG

porare durch kurze (500–1000 msec) Reiztone re-  
 frequenz (2000–3000–4000 Hz) verursachte Schwell-  
 en weisen bei vergleichbaren experimentel-  
 Bedingungen ein vollkommen analoges Verhalten  
 diejenigen nach längeren Stimulationen erhaltenen.  
 Die maximalen Werte solcher Schwellenerhöhungen  
 andern zeigen sobald der Reizton ein genügend  
 (100 dB SPL) Intensitätsniveau erreicht hat, eine  
 liche Tendenz zur Verschiebung nach einer Fre-  
 quez die etwa eine halbe Oktave höher als die Frequenz  
 Reiztones liegt. Auch bei unseren Befunden konnte  
 diese Tendenz besonders klar und konstant nach-  
 esen werden nur wenn die Schwellenerhöhung  
 nen Reizton von 3000 Hz verursacht worden war.  
 nenveränderungen zeigten bei den verschiedenen  
 bedeutsame quantitative individuelle Varia-  
 tionen. Kurze Tonreize können mit einigen Vorteilen  
 gewinn Genauigkeit der Messungen) der weiteren  
 schungen über Nacheffekte starker akustischen  
 ze angewandt werden. Eine Erklärung über die Be-  
 tung individueller Variationen konnte klinische An-  
 genen mit sich bringen.

## REFERENCES

- 1953 *Investigations on Short Tones*. Univer-  
 sity of Aarhus.  
 W & Robinson D W 1970 An investigation of  
 the Effects of Occupational Noise on Hearing. In

*Sensorineural Hearing Loss* p 177 J & A Churchill  
 London.

- Davis H, Morgan C T, Hawkins J E jr, Galambos  
 R & Smith F W 1950 Final report on temporary  
 deafness following exposure to loud tones and noise.  
*Acta Otolaryngol* (Stockh) Suppl 88.  
 Elliot H N & Fraser W 1970 Fatigue and Adapta-  
 tion. In *Foundations of Modern Auditory Theory* (ed  
 J Tobias) p 117 Academic Press New York.  
 Klyn M 1960 Temporary threshold shift and auditory  
 trauma following exposure to steady state noise. *Acta  
 Otolaryngol* (Stockh) Suppl 152.  
 Larsen B 1939 Investigations of professional deafness in  
 shipyard and machine factory labourers. *Acta Oto-  
 laryngol* (Stockh) Suppl 36.  
 Ward W D, Glorig A & Sklar H L 1958 Dependence  
 of temporary threshold shift at 4 kc on intensity and  
 time. *J Acoust Soc Am* 30 944.  
 — 1959 Temporary threshold shift from octave band  
 noise: applications to damage-risk criteria. *J Acoust  
 Soc Am* 31 322.  
 Zwislock J 1950 Theory of the acoustical action of the  
 cochlea. *J Acoust Soc Am* 22 778.  
 Zwislock J & Prodda E 1952 On the adaptation  
 fatigue and acoustic trauma of the ear. *Experientia* 8  
 279.  
 E Prodda M D  
 Dept of Otolaryngology  
 University Hospital  
 Bologna  
 Italy

## VASCULAR HISTOLOGY OF THE GUINEA PIG COCHLEA

A. Axelsson and D. Vertes

*From the Department of Otolaryngology, Sahlgren's Hospital University of Göteborg, Sweden*

(Received March 4 1977)

**Abstract** Detailed histological findings on all the regularly occurring cochlear vessels in the normal guinea pig are presented. Vessels were examined using a surface preparation technique and phase contrast microscopy. Particular attention was paid to the density of red blood corpuscles, the thickness of the vascular wall, the occurrence of endothelial cells and pericytes, together with their possible influence on the vessel lumen and the existence of perivascular spaces and vessels lacking blood corpuscles. The data are compared with some previous findings of 'vascular pathology' following noise stimulation. The possible functional significance of some of the morphological findings is also discussed.

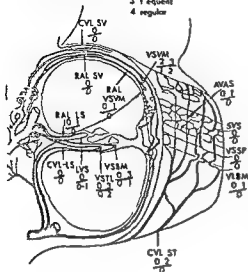
The study of the cochlear blood supply is sometimes limited by its complexity and inaccessibility. The location of vascular pathology, for example, subject to change, depending on individual susceptibility differences, variability between different vessel systems, as well as differences in the vasculature between the cochlear turns. Although this variability seems to suggest a need for systematic investigations in which all cochlear vessels in control animals as well as in experimental animals are examined, a review of the literature reveals a lack of such studies. It is not surprising, then, that the role of the blood supply in noise induced hearing loss is not clearly understood. Some authors reportedly found a decrease in red blood cells in the vessels below the basilar membrane (Lawrence et al., 1967, Lipscomb & Roettger 1973) and in the stria vascularis (Lawrence, 1972) following noise exposure. Presumably related to this

was the presence of swollen endothelial cells partially occluding the vessel lumen. Both endothelial cells and pericytes are thought to be involved in the regulation of blood flow (Rhodin, 1968, Kellerhals 1971, Kimura & Ota, 1974), the mechanism of regulation is still disputed. Kellerhals (1971b) while noting no changes in the spiral lamina capillary networks, found some striae packed with red blood cells following exposure to impulse noise. Duvall et al. (1971) sacrificed animals at various intervals following noise exposure, they reported changes in the stria vascularis ranging from constricted and collapsed vessels containing few red blood corpuscles, to dilated vessels filled with distorted red blood corpuscles. The density of red blood cells, whether packed or widely spaced, could also be a result of the preparation technique (Lawrence, 1972, Axelsson 1976). Fried et al. (1976) exposed chinchilla to intense low frequency octave band noise. In most of their animals they found one or two areas of complete degeneration of the stria vascularis. They found inconsistencies in location and extent of such damage. Using similar low frequency octave band noise as Böhne (1976) reported minimal damage to blood vessels beneath the maximally damaged

This study was supported in part by the Swedish Board for Technical Development (No. 76-3998) and the Environmental Protection Fund (74/42).

Fig. vascular spaces/  
Vessels lack fig  
Blood Co particles

Occurrence  
0 no  
1 seldom  
2 occasional  
3 frequent  
4 regular



RED DYE

OCULUM AND ASSOCIATED VESSELS

PER LYMPH AND ASSOCIATED VESSELS

1 One cochlear turn apico-basal section showing early occurring vessels their relationship to cochlear spaces and the existence of pervascular spaces (PVS)

RAA radiating arterioles—scala vestibuli basally  
SV collecting venules—scala vestibuli VSM the vessel at the vestibular membrane AVAS arteriovenous anastomoses SVS stria vascularis VSSP the vessel of the spiral prominence VLBW the venules at the basilar lamina CVL-ST collecting venules—scala tympani  
LS radiating arterioles—spiral lamina CVL-LS collecting venules—spiral lamina LVS limbus vessels  
L the vessel of the tympanic lip VSBM the vessel of the basilar membrane

ions of the organ of Corti but the condition of other vessels throughout the cochlea not described. Thus, although the blood flow is increasingly thought to have an influence in various types of sensorineural hearing losses, its influence in noise trauma is controversial. Too often results have been in terms of representing pathology—without a basis of normative data with which the results could be compared. It is the purpose of this study to begin to

supply some of that normative data by which vascular pathology, regardless of cause, might be assessed.

## MATERIAL AND METHODS

Seven normal young healthy guinea pigs were used for the experiments. The animals were sacrificed without anesthesia by decapitation with sharp scissors. The temporal bones were immediately removed, the bullae opened and the cochlea assessed according to a previously described method (Axelsson et al., 1974, 1975). Briefly, the cochlea was fixed by slowly injecting 5% glutaraldehyde through an apical opening and through the oval window from which the stapes had been removed. After 24 hours in the fixative the cochlea was decalcified in 5% EDTA which was changed daily until the decalcification process was complete (about 7 days). An apico-basal section of the cochlea was made and all structures were examined for gross changes. The cochlea was then counterstained in 0.05% osmic acid for 10 minutes, dehydrated in an alcohol series of increasing concentration and stored in glycerol. In one animal the osmic acid counterstaining of both ears was omitted in order to evaluate the influence of this stain on cochlear vessels. After further dissection cochlear specimens were examined by phase contrast microscopy, usually under 250–1200× magnification. The following regularly occurring cochlear vessels were examined in all half turns of each cochlea of each animal.

### External wall

Scala vestibuli  
radiating arterioles  
collecting venules  
the vessel at the vestibular membrane  
the vessel of the scala vestibuli

Scala media  
radiating arterioles  
arteriovenous anastomoses  
stria vascularis  
the vessel of the spiral prominence

*Scala tympani*

the venules at the basilar membrane  
collecting venules

*Spiral lamina*

radiating arterioles  
collecting venules  
the limbus vessels  
the vessel of the basilar membrane  
the vessel of the tympanic lip

A schematic diagram of these vessels including their relation to the cochlear lymph is shown in Fig. 1. In all these vessels particular attention was paid to the following: (1) the distance between red blood corpuscles (RBC), (2) the thickness of the vascular wall, (3) the occurrence and size of pencytes (PC) and endothelial cell nuclei, hereafter referred to as endothelial cells (EC), and their possible influence on the vessel lumen, (4) the existence of so called perivascular spaces (PVS) and vessels lacking blood corpuscles (VSLBC) the formerly called avascular channels, and (5) the occurrence of pigment granules, melanocytes or any other regularly present extracellular substance in the stria bed.

## RESULTS

*Radiating arterioles of the Scala vestibuli (RAL-SV)*

The radiating arterioles of the external wall were studied apically where the scala vestibuli merges with the scala tympani of the next turn. This is the region immediately peripheral to the so called glomeruli, the winding parts of the RAL with a spring coil appearance. The number of RAL in this region is high, particularly in the basal turn. The RAL run radially over the scala vestibuli to supply all capillary areas in the external wall. Red blood corpuscles (RBC) appeared in the lumen in several rows and were situated close to each other. The vessel wall was always thick. Due to this thickness, endothelial cells (ECs) and

pencytes (PCs) were difficult to observe. Occasional PCs were observed particularly in the second and fourth turn. It appeared to be of normal size and had no influence on the vascular lumen. Occasional perivascular space or vessel blood corpuscles could be observed in the fourth turn of the cochlea, but this was an unusual finding.

*Radiating arterioles of the Scala basally (RAL-VSVM)*

The radiating arterioles of the scala were also examined in the basal part of the external wall at the level of the vessel vestibular membrane (VSVM). The lumen in all turns was filled by RBCs. They lay close to each other and were oriented in different planes. Though still of considerable size, the vascular wall was thinner than apically in the scala vestibuli, especially in the apical turns. A slim layer of substance was often seen on the outside of the vessel. In many preparations ECs could often be observed in the RAL at this level as a regular finding. Occasional PCs most commonly at the ramification of the vessel were observed with a somewhat increasing frequency apically in the cochlea. Perivascular spaces were demonstrated in the three basal turns with a fairly low frequency. There seemed to be a gradual transition from thick muscled walled vessels to those with perivascular spaces and thinner walls containing fewer elements. The occasionally seen vessel lumen blood corpuscles was most common in the branches connecting VSVM. In general neither ECs or PCs appeared to influence the vessel lumen.

*Collecting venules of the Scala vestibuli (CVL-SV)*

Occasional CVL run spirally in the most peripheral parts of the scala vestibuli at the junction of two cochlear turns. More peripherally in the scala vestibuli CVL often turn off at right angles and run radially and parallel



Fig 2 Guinea pig 4th turn external wall. The capillaries of the stria vascularis (SVS), the vessel at the vestibular membrane (VSV) and the vessel of the spiral prominence (VSSP) and a vessel lacking blood corpuscles (VSLBC) are seen. The enclosed portion of VSV is seen at a higher magnification in Fig 3. Arrows = remnants of dark material within VSLBC and the so-called blebs in SVS.

L. In general, RBCs were situated close to each other and seemed to course easily through the lumen. Occasionally, the lumen was narrower and blood cells were obliquely or longitudinally. CVL had considerably thinner vascular wall than L. Slim to normal sized ECs and normal-sized PCs without influence on the vascular wall were a fairly frequent finding in all cochlear turns. In general, no perivascular spaces or vessels lacking blood corpuscles were demonstrated.

Vessel at the vestibular membrane (VSV)

VM shown in Figs 2-4, is a capillary vessel belonging to the capillary area apical to the

attachment of the vestibular membrane in the external wall. This capillary area is bordered by the vessel of the scala vestibuli (VSSV) apically and by the vessel at the vestibular membrane (VSV) basally. Usually RBCs of VSV were situated close to each other and were situated longitudinally in the lumen. Though less frequent, obliquely or transversely oriented RBCs were also seen as shown in Figs 2 and 3. In general, the lumen appeared light, though a darkened lumen was occasionally seen. It was not possible to determine if this was due to ECs or to other changes in the vessel wall or within the lumen. The vessel wall appeared thin. ECs were common and of normal shape. Sometimes, as in Figs 3 and 4, they appeared to influence

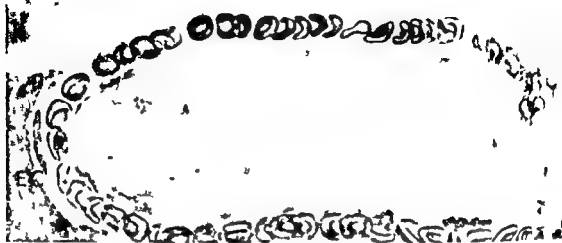


Fig 3 Guinea pig 4th turn vessel at the vestibular membrane (VSM). Well-defined perivascular spaces (PVS) endothelial cells (ECs) and pericytes (PCs) are observed

Note the change in orientation of red (→) at the site of an EC juxtaposed with a PC

the vascular lumen. Normal sized PCs appeared occasionally in the basal turn and at times in the apical turn. Regardless of size PCs by themselves tended to have no diminishing effect on the vessel lumen. Perivascular

spaces were a regular finding but were sometimes absent or minimal towards the cochlear base. In no vessel other than VSM and in no vessel of the basilar membrane were the perivascular spaces so easily observed. Mostly



Fig 4 Guinea pig 3rd turn vessel at the vestibular membrane (VSM). Upper vessel section shows that blood corpuscles are situated some distance apart and are easily mistaken for a vessel lacking blood corpuscles. Arrow: EC and adjacent pericyte appear to influence the vessel lumen.

ared as in Fig 3, as a smooth, double out without any pursings. Vessels lacking d corpuscles were also common in all s and appeared to be in direct commu- tion with the perivascular spaces. Most of vessels lacking blood corpuscles had a hazy grey and empty appearance (see 2). Sometimes, however, as is also shown in Fig 2, there seemed to be dark material in what had previously been the lumen. Distensions from vessels lacking blood cor- puses to fibrous strands were uncommon. Usually the lack of clear contrast from the surrounding tissues made vessels lacking blood corpuscles with such a fibrous organization difficult to discern.

#### *Vessel of the scala vestibuli (VSSV)*

The vessel of the scala vestibuli, VSSV, which constitutes the apical margin of the capillary of the scala vestibuli, appeared similar to VSM. RBCs, however, were at times widely spaced, lying more than 5  $\mu$ m from each other. Vascular spaces and slim PCs were sometimes present.

#### *Arterioles of the SVS (SVS)*

Arterioles of the SVS (see Fig 2) showed a variation of all cochlear vessels between animals in the same animal and between animals. The capillaries were always packed with RBCs. The vascular wall appeared very thin and no perivascular spaces were found. The diameter of the capillaries varied greatly and sometimes segments of the capillaries would be seen narrowing down and being trans- formed into a vessel lacking blood corpuscles. Generally neither PCs nor ECs could be observed, though on rare occasions a long, thin stretched EC or some kind of deposit was seen in the walls of the capillaries. Blebs or small circular empty spaces 5-6  $\mu$ m in diameter seen in Fig 2, were sometimes seen in the vessel lumen in the second to fourth turns. Minute granula were often oriented along vessels in all turns. Larger clumps of pigment were also a common finding through-

out the cochlea. Occasionally, melanocytes and pigment clusters, transitional forms of melanocytes, were seen. Granula, pigment clumps and melanocytes all appeared to be more frequent apically. As seen in cross section, it appeared that the pigment clumps were most deeply situated in the stria vascularis, with a layer of fine granula more superficially and a mixture of pigment clumps, fine granula and melanocytes closest to the SVS surface.

#### *The vessel of the spiral prominence (VSSP)*

VSSP (see Fig 2) lies deep in the spiral prominence and consequently was less easily observed than many other vessels. RBCs were situated close to each other, passing the lumen transversely and occasionally obliquely or longitudinally. The vessel wall appeared thin and sometimes the vessel seemed to be surrounded by a thick tissue strut or collar. Perivascular spaces were not regularly found but what appeared to be a narrow perivascular space could at times be demonstrated in the basal turn. Vessels lacking blood corpuscles were not demonstrated. Though improving somewhat apically, ECs and PCs were typically difficult to observe. When they were seen, they appeared to be small and slim. PC influence on the vessel lumen could not be determined. It appeared, however, that ECs often influenced the vascular lumen in that the blood corpuscles at these sites took a longitudinal instead of a transverse course. Melanocytes without any direct relation to VSSP appeared in all turns but clearly less often in the basal turn than in the others.

#### *Radiating arterioles of the scala media (RAL-SM) and arteriovenous anastomoses (AVAS)*

Branches of RAL-SV supply all kinds of capillary areas in the external wall of the cochlea and lie external to the capillary vessels of the scala vestibuli and scala media. Many branches run directly over to the collecting venules of the scala tympani without supplying any of the capillary areas. These vessels ap-

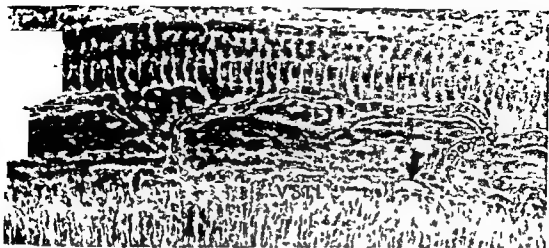




Fig 3 Guinea pig 1st turn of venule scala tympani. Pock-like perivascular spaces ( $\Rightarrow$ ) and pericytes (PCs) are seen. Blood cell ton within lumen seemingly abut endothelial cells ( $\rightarrow$ )

appear to constitute some kind of by pass mechanism and are here termed AVAS. This term while signifying that these vessels connect arterioles with venules may be incorrect from a histological point of view. In view of their proximity to the SVS Fig 1 depicts the AVAS as endolymph facing vessels. However based on evidence that the basal cells of the stria probably provide a tight seal between stria and spiral ligament vessels (Kimura & Schuknecht 1970; Smith 1973; Hawkins 1973) it is much more likely that the AVAS are associated with perilymph. Blood corpuscles

within the lumen could be situated close to each other or some microns apart. Blood RBCs were most often oriented transversally within the lumen and apically most often longitudinally. Due to their location deep within the spiral ligament the existence of perivascular spaces (PCs and ECs) was difficult to observe. Perivascular spaces were an unusual feature; sometimes it appeared that they were lacking in large calibre AVAS while present in vessels of more delicate size. Vessels lacking pericytes were never seen in the stria. When observable ECs appeared fair



Guinea pig 1st turn the vessel of the tympanic (STL) and the vessel of the basilar membrane (VSBM). The density of red blood corpuscles in VSBM is to vary from close to spaced. Perivascular spaces

( $\Rightarrow$ ) and vessels lacking blood corpuscles ( $\Leftarrow$ ) are seen in both vessels. Channel like system of bridges between perivascular spaces and vessel lacking blood corpuscles in VSBM ( $\Leftarrow$ ) are seen.

PCs infrequent, both of normal size and without any influence on the vascular lumen.

#### *Collecting venules of the scala tympani (L-ST) and venules at the basilar membrane (VLBM)*

(see Fig 5) drain all capillary areas in external wall. They also connect directly to the AVAS, particularly in the basal turn. CVL constitute the only vessels in the scala tympani of the external wall. At the attachment of the basilar membrane, CVL make an S-shaped loop and then, more basally, they run spirally for a short distance. These vessels, when viewed perpendicularly, seem to constitute spirally running vessels, the venules of the basilar membrane (VLBM). The histology of VLBM is very difficult to study because of their location deep in the spiral ligament. The observations were similar to those of the CVL-ST. The RBCs in the lumen of the CVL were situated close to each other and were oriented transversely. In larger vessels there was more than one column of RBCs often with obliquely or longitudinally oriented RBCs. It was very difficult to determine if there were perivascular spaces in these vessels and there was a great deal of individ-

ual variation. As seen in Fig 5, straight perivascular spaces similar to those of the vessel at the vestibular membrane and the vessel of the basilar membrane were not seen. In the two basal turns there appeared to be some kind of pocket like irregular spaces surrounding CVL, particularly at the basal part of each scala tympani. Apically, it was more common to find CVL without any perivascular like structures. Vessels lacking blood corpuscles were never observed. Normal sized PCs without any influence on the vascular lumen were a common finding. As opposed to other regions of the cochlea PCs often appeared to have a triangular shape (see Fig 5). The frequency of PCs seemed highest in the middle region of the cochlea and less frequent towards the base and apex. ECs of normal appearance and without influence on the vessel lumen were also a regular finding. As shown in Fig 5 occasionally an EC juxtaposed with a PC was seen to influence the vessel lumen.

#### *Radiating arterioles and collecting venules of the spiral lamina (RAL, CVL-LS)*

These vessels originate from the same central parts of each cochlear turn and radiate over the spiral lamina. They are fairly easy to dif-



Fig. 7 Guinea pig 2nd turn the vessel of the tympanic lip (VSTL) and the vessel of the basilar membrane (VSBM). Granula surround VSBM. Perivascular spaces

(==) are present in VSTL but are lacking in VSBM. Endothelial cell appears to diminish vessel lumen (→).

ferentiate when contrast injected but impossible to separate with the present technique. In addition, these vessels are very hard to discern due to the osmic acid stained nerve fibres which obscure the view. In one animal in which the cochlea was unstained, these vessels were easier to observe. RBCs were transversely situated in the lumen. There appeared to be large perivascular spaces in many areas, no vessels lacking blood corpuscles and frequent PCs and ECs of normal appearance. Although EC influence on the vessel lumen could not be determined, sometimes a large PC appeared to reduce the lumen at the level of attachment to the vestibular membrane.

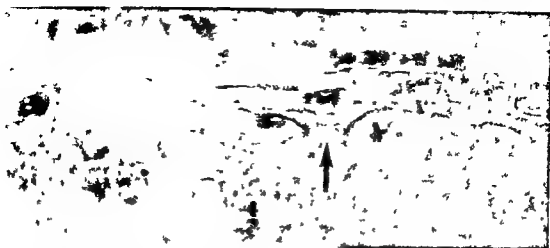
#### *The limbus vessels (LVS)*

LVS are made up of capillary arcades in the spiral limbus and are the only vessels of the spiral lamina which face endolymph (see Fig. 1). They are situated fairly deeply in the tissue and consequently it is difficult to get a close view of them. Most commonly RBCs in the lumen lay close together but more

widely spaced RBCs could also be seen. blood cells were transversely oriented in lumen or, less commonly obliquely. In several perivascular spaces and vessels blood corpuscles were not demonstrated. When found, vessels lacking blood corpuscles tended to be in the basal parts of the cochlea. PCs were an infrequent finding, their was generally normal, but in some cochleas they were slim. They did not influence vessel lumen. ECs of normal appearance with no diminishing effect on the lumen were frequently seen.

#### *The vessels of the basilar membrane (VSBM)*

VSBM, also called vas spirale or the spiral vessel, is shown in Figs 6-8. This is an interesting vessel since it is most closely situated to the organ of Corti (OOC) and is regularly found in man as well as in guinea pig. In other mammals it appears to be rudimentary and is only occasionally demonstrated (rhesus monkey, chinchilla) or is



\* Guinea pig 2nd turn the vessel of the basilar (VSBV) The density and orientation of red

blood corpuscles are seen Arrows—tube like openings from perivascular spaces (PVS)

all (rabbit) In the guinea pig, VSBM most easily observed in the second half first turn. The diameter of VSBM appeared to vary appreciably and consequently density of the RBCs also varied. In general, RBCs were situated close to or within microns of each other. Occasionally, there would be larger segments of the vessel with much greater separation between RBCs. RBCs were almost invariably situated normally in the lumen and very seldom transversely or obliquely. Perivascular spaces were regularly demonstrated in the basal, second and third and only occasionally in the third and fourth cochlear turns. Compare, for example, Figs 6 and 7 showing turns 10 and 15, respectively. Vessels lacking blood corpuscles were not a frequent finding in any turn but were most often demonstrated in the first and least often in the third turn. PCs were more or less regularly found and were particularly more common in the apical two turns of the cochlea. PCs of larger than normal size were observed in all except the basal turn with fairly equal distribution. PCs did not appear to influence the vessel lumen diameter. ECs were a frequent finding in all cochlear turns. Though not usual, ECs in the two basal turns appear larger than normal (as in Fig 7)

and cause a slight depression in the vascular wall. Granulated material, also seen in Fig 7, regularly surrounded VSBM particularly in the two basal turns. The origin or character of these granula is unknown, however they were not as easily observed in the unstained animal thus indicating that they may be osmiophilic in character. As is seen most clearly in Fig 6, perivascular spaces often formed a separate, almost channel like system of bridges and open spaces together with the vessels lacking blood corpuscles. A finding most common in the first turn which has not previously been demonstrated was what appeared to be openings like small tubes from these perivascular spaces into the tissues (see Fig 8). These appeared both on the peripheral and on the central side of VSBM but were more common on the former. ECs on the vessel wall opposite to the perivascular tube openings were common.

#### *The vessel of the tympanic lip (VSTL)*

As shown in Figs 6 and 7, osmic acid darkens the myelinated nerve endings, thus making VSTL or the inner spiral vessel, difficult to observe in preparations treated with the present technique. Though RBCs were most commonly situated close to each other, there

large variations and often wider-spaced RBCs were seen. In general, RBCs were oriented longitudinally in the lumen, occasionally obliquely or transversely. Perivascular spaces were always found in the basal turn and decreased apically. The perivascular spaces often appeared to be larger than those surrounding VSBM. Short segments of vessels lacking blood corpuscles were occasionally demonstrated. PCs, though not abundant, were demonstrated with increasing frequency in the apical turns. ECs which were less frequently seen on this vessel than on VSBM, sometimes appeared to influence the vascular lumen. The granulated material surrounding VSBM was in general not demonstrated around VSTL but sometimes followed an interconnection between the two vessels and continued for a short distance around the VSTL.

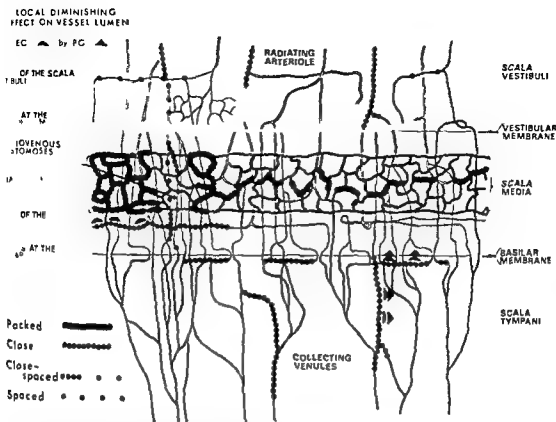
## DISCUSSION

The advantages and disadvantages of our modified method of viewing the vasculature have been described previously (Axelsson et al., 1974, 1975). We are aware that the present technique may influence the cochlear vessels in that circulation and vessel function does not stop instantaneously with the death of an animal. We are equally aware that this technique results in a static view of a normally dynamic process. Such limitations, however, are inherent in all presently available techniques.

Some cochlear vessels are highly variable between animals, while others, notably the capillaries of the stria vascularis, appear similar in different individuals. Vessels and related vascular phenomena can also vary between ears in the same animal, between turns of a single cochlea, and between sections of the same turn. The descriptions which follow, therefore, represent the generalized findings of a large number of observations. Exceptions and variations, however, may and do occur—thus the data presented should not be thought of as definitive. In addition, most of the ob-

servations described here are 'visual impressions', and, as such, cannot conclusively determine the existence of, for example, perivascular spaces and/or vessels lacking blood corpuscles. Obviously, other histological techniques employing cross-sectional views of vessels are necessary for these types of determinations.

A summary of cochlear vessels facing perilymph and those facing endolymph is shown in Fig. 1. This figure also shows interestingly that perivascular spaces are most commonly found and most regularly occur in vessels facing perilymph. These vessels include the following: Radiating arterioles of the scala vestibuli basally, collecting venules of the scala vestibuli, the vessel at the vestibular membrane, the vessel of the scala vestibuli radiating arterioles and collecting venules of spiral lamina, the vessel of the basilar membrane and the vessel of the tympanic membrane. Those vessels facing perilymph which do have regular or frequent perivascular spaces are radiating arterioles of the scala vestibuli apically, with their thick vascular walls, the sparsely occurring collecting venules of the scala vestibuli. Perivascular spaces are also irregular and/or infrequent in the following: the vessel of the spiral prominence, arteriovenous anastomoses and the lymphatic vessels. We feel that the occurrence of perivascular spaces in vessels facing perilymph and not in those facing endolymph must be of functional significance. The most probable explanation would be that these spaces contain some kind of intermediate fluid between blood and perilymph. It has been speculated (Maggio, 1966; Schindler & Schneider, 1966) that the radiating arterioles of the scala vestibuli are one possible source of perilymph production. The fact that the radiating arterioles of the scala vestibuli apically have few regular perivascular spaces does not, of course, refute this. It does suggest, however, that perilymph transfer and absorption is unlikely to occur in the radiating arterioles there peripherally. In general, no opening c-



9 One cochlear turn external wall, showing the density of red blood corpuscles and the influence of endothelial cells (EC) and pericytes (PC) on the vessel lumen

found on the perivascular spaces at the highest magnifications. Only in the vessel of the basilar membrane were there sometimes tube-like openings (see Fig. 8) on both the central and more frequently the peripheral side. These give the visual impression of an 'hose' pouring perivascular fluid out in the spaces and fluids nearby, i.e. myelinated nerve endings, the organ of Corti and perilymph. Alternatively, these hose-like structures could be fibrous strands of vessel walls. Primarily, it is beyond the scope of this study to determine the exact nature of these perivascular elements. Conclusive evidence must await electron microscopic investigations. With the exception of an occasional occurrence in the limbus vessels, vessels lacking red blood corpuscles were also only found in the 'open' vessels which face perilymph the

vessel at the vestibular membrane, the vessel of the basilar membrane and the vessel of the tympanic lip (see Fig. 1). Particularly in the spiral lamina, there is a clear communication between the perivascular spaces and the vessels lacking blood corpuscles. As is shown in Fig. 6, it appears that the vessels lacking blood corpuscles, in some cases, are part of an extraluminal vascular fluid system. Contrarily, the hazy grey vessels lacking blood corpuscles in the scala vestibuli of the external wall (Figs. 2 and 4) appear more as possibly degenerated vessels. The ultimate question whether vessels lacking blood corpuscles function in the circulation of a non-corpuscular fluid such as plasma or are closed-down vessels cannot be solved by the present technique.

As stated in the introduction of this paper, the previously termed 'avascular channels'

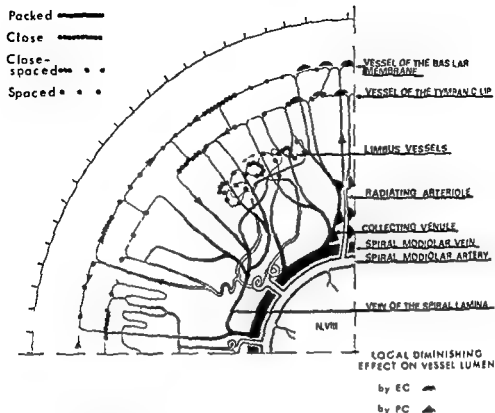


Fig. 10 One cochlear turn spiral lamina showing the density of red blood corpuscles and the influence of

endothelial cells (EC) and pericytes (PC) on the vessel lumen

have here been called 'vessels lacking blood corpuscles'. The term avascular indicates that there is no circulation of blood elements. The absence of a vessel without RBCs does not necessarily indicate such a state, since this vessel or vessel section may be circulating either plasma (plasma skimming) or serum. Another possibility is that there is a momentary or somewhat longer interference with this vessel or vessel section because of a transient blockage by a red or white blood corpuscle. These channels are certainly vascular, though with the present techniques it cannot be stated whether there is circulation in them. Consequently we would prefer the somewhat more descriptive 'vessels lacking blood corpuscles'. Hawkins, who termed these vessels 'avascular channels' (1967), presumably sought to describe a possible degenerative phenomenon of the actual vessel by this term. Again, since they are found in the normal animal, they may

not indicate a degenerative phenomenon, but rather be a normal finding.

The stria vasculans (Smith 1957, Magi 1966) and less frequently the limbus vessel (Cimino & Grisanti 1967, Voldrich 1969) have been thought to be involved in endolymph production and/or absorption. The fact that of the spiral lamina vessels the limbus vessels are the only ones facing endolymph would seem to be further indirect evidence of their possible role in endolymph transfer.

The density of RBCs in the various vessels ranged from compact (packed) to spaced (100  $\mu\text{m}$  apart) as shown in Table I. The RBC density for each vessel and vessels with lumen influenced by EC and/or PC is shown schematically in Figs 9 and 10 (See also Figs 4, 5 and 7). Most importantly it can be seen from these figures that in the normal guinea pig one can expect the density of RBCs in vessel to vary with or without corresponding ECs or

# le I *The density of red blood corpuscles chlear vessels*

vascularis

- "
- ating arterioles—scala vestibuli apically
- ating arterioles—scala vestibuli basally
- lecting venules—scala vestibuli
- vessel III the vestibular membrane
- vessel of the spiral prominence
- lecting venules—scala tympani
- venule at the basilar membrane

re-Spaced

- enous anastomoses
- bus vessels
- vessel of the basilar membrane
- vessel of the tympanic lip

ed

- vessel of the scala vestibuli

's affecting the vessel lumen. As previously  
 tioned, some authors (Lawrence et al.,  
 7, Lipscomb & Roettger, 1973) found a de-  
 n RBCs in the vessels below the basi-  
 membrane following noise exposure. The  
 sence of swollen endothelial cells affecting  
 el diameter in the basilar membrane ves-  
 ls (Hawkins, 1971, Lipscomb & Roettger,  
 73) and in stria vessels (Lawrence, 1972)  
 also reported in noise damaged ears. As  
 in Fig. 9, in the normal guinea pig the  
 , of RBCs in the vessel of the basilar  
 a ranges from close to widely-  
 As is also seen, ECs can sometimes  
 luence the vessel lumen. On the contrary,  
 ig 10 shows that RBCs in stria vessels are  
 ually quite compact. ECs and PCs are diffi-  
 cult to observe in the stria vascularis but  
 seen do not seem to affect the dia-  
 of the capillaries of the stria bed. These  
 eliminary findings suggest that the presence  
 widely spaced RBCs in conjunction with  
 swollen ECs partially occluding the vessel  
 n in the vessel of the basilar membrane  
 following noise exposure cannot be viewed  
 a pathological finding since they are present  
 normal animals as well. Similar changes in  
 he stria vascularis of noise exposed animals

are more likely to be pathological, since they  
 were not found in our control material. In all  
 probability, vascular pathology, regardless of  
 cause, will not be manifested by qualitative  
 differences between animals but rather by  
 quantitative differences. This fact, however,  
 can only be determined by further in-depth  
 investigations.

We have previously noted a simplification in  
 the vasculature apically (Axelsson 1968). In  
 this study perivascular spaces in the spiral  
 lamina vessels were found to be approximately  
 twice as frequent in the two basal as in the  
 two apical turns. In addition, the existence of  
 granula surrounding the vessel of the basilar  
 membrane was more prevalent in the three  
 basal turns than in the apex. The granula,  
 which could be a by product of high ATPase  
 activity, and the prevalence of perivascular  
 spaces may be related to the previously dem-  
 onstrated high metabolic rate in the cochlear  
 base (Nakai & Hilding, 1967). Interestingly,  
 in the external wall vessels, perivascular  
 spaces were distributed fairly evenly through-  
 out the cochlea. Earlier the hypothesis was  
 advanced that perivascular spaces (and less  
 frequently vessels lacking blood corpuscles)  
 may be involved in the processes of perilymph  
 transfer and absorption. The differences in the  
 distribution of perivascular spaces would seem  
 to suggest that this function could be better  
 carried out basally than apically, at least in the  
 spiral lamina.

In summary it should be restated that the  
 vascular conditions described in this paper are  
 for the guinea pig. Preliminary studies of the  
 chinchilla suggest that differences can (and  
 do) occur between species. We are reluctant  
 to speculate, for example, on the significance  
 of the blebs seen in Fig. 2 in the stria vas-  
 cularis, since similar phenomena have not  
 been observed in the chinchilla. It is sug-  
 gested, therefore, that these initial results be  
 regarded with caution and that they should  
 be substantiated, if possible, with further  
 quantitative investigations of a similar na-  
 ture.



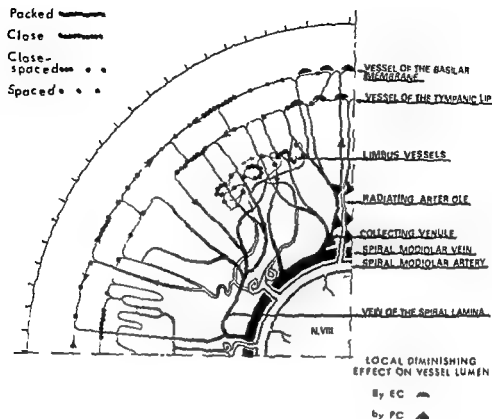


Fig. 10 One cochlear turn spiral lamina showing the density of red blood corpuscles and the influence of

endothelial cells (EC) and pericytes (PC) on the vessel lumen

have here been called 'vessels lacking blood corpuscles'. The term avascular indicates that there is no circulation of blood elements. The absence of a vessel without RBCs does not necessarily indicate such a state, since this vessel or vessel section may be circulating either plasma (plasma skimming) or serum. Another possibility is that there is a momentary or somewhat longer interference with this vessel or vessel section because of a transient blockage by a red or white blood corpuscle. These channels are certainly vascular, though with the present techniques it cannot be stated whether there is circulation in them. Consequently we would prefer the somewhat more descriptive 'vessels lacking blood corpuscles'. Hawkins, who termed these vessels 'avascular channels' (1967), presumably sought to describe a possible degenerative phenomenon of the actual vessel by this term. Again since they are found in the normal animal they may

not indicate a degenerative phenomenon rather be a normal finding.

The stria vasculans (Smith, 1957, Magi, 1966) and less frequently the limbus vessel (Cimino & Grsanti, 1967, Voldrich, 1969) have been thought to be involved in endolymph production and/or absorption. The fact that of the spiral lamina vessels the limbus vessels are the only ones facing endolymph would seem to be further indirect evidence of their possible role in endolymph transfer.

The density of RBCs in the various vessels ranged from compact (packed) to spaced (10  $\mu$ m apart) as shown in Table I. The RBC density for each vessel and vessels with lumen influenced by EC and/or PC is shown schematically in Figs 9 and 10. (See also Figs 5 and 7.) Most importantly it can be seen from these figures that in the normal guinea pig one can expect the density of RBCs in vessels to vary with or without corresponding ECs

## COCHLEAR PATHOLOGY FOLLOWING EXPOSURE TO MERCURY

M Anniko and L Sarkady<sup>1</sup>

*From the Department of Otolaryngology, Karolinska sjukhuset and King Gustaf V Research Institute  
Karolinska Institutet Stockholm Sweden*

(Received March 17 1977)

**Abstract** The sensory and secretory epithelia may become morphologically changed following the exposure to

mercury (auditory evoked potentials and electron micrograph studies). Acute intoxication mostly affected both afferent and efferent nerve terminals and the hair cells while chronic poisoning could also damage the stria vascularis.

Since the classic paper published by Hunter et al in 1940 on mercury intoxication, many investigators (Takeuchi, 1970, Lofroth, 1969, Kurland et al 1960) have described the toxicology of mercury compounds. There have been reports from all parts of the world concerning poisonings and even deaths resulting from the ingestion or inhalation of mercurials or from cutaneous contact with these compounds (Troen et al, 1951, Jalili & Abbasi 1961, Friberg 1971, Rosen et al, 1966). Comparatively few reports of neuro-otological observations following intoxication by mercurials (Nosaka et al, 1970, Fujisaki et al 1971) have been published describing dys-equilibrium and impairment of hearing and speech discrimination as the main findings.

According to the observations of Mizukoski et al (1975) the neuro-otological disturbances following chronic mercury intoxication may be considered mainly as lesions of the retro-cochlea and of the oculomotor system in the brain stem and the cerebellum.

To our knowledge the only animal experimental study on the effect of mercury on the inner ear was performed by Falk et al (1974) who described the degeneration of outer hair cells of the organ of Corti mainly at 2½ turns from the base of the guinea pig cochlea.

The aim of the present study was to investigate morphologically the effect of inorganic mercury on the inner ear structures. The present paper deals with the cochlear part of the labyrinth and the effects on the vestibular part of the inner ear will be evaluated in a separate paper.

### MATERIALS AND METHODS

Sixty two young healthy guinea pigs (250-350 g) were treated with daily subcutaneous injections of mercury chloride ( $HgCl_2$  as a 11.5% solution in sterile water) in doses ranging from 2.5 to 25 mg/kg b.w.

The total dose ranged from 111 to 90 mg/kg b.w. administered during 1-49 days. The survival time after the last injection varied from

Supported by grants from Karolinska Institutet and the Swedish Medical Research Council (grant no. 17X-00720-12B).

<sup>1</sup>Then visiting scientist at the Department of Otolaryngology Karolinska sjukhuset and King Gustaf V Research Institute. Present address: Department of Otolaryngology Semmelweis University Clinic Budapest Hungary.

1 day to 1 month. The control group of animals consisted of 9 young untreated guinea pigs.

The animals were weighed daily and examined for general appearance and activity. The gross neurologic investigation included gait disturbance, hindlimb crossing phenomenon and the presence/absence of Preyer's reflex.

### Morphological procedures

The cochlea was investigated by light and electron microscopy for both qualitative and quantitative analysis of cochlear damage. Specimens were taken according to the technique of Wersäll (1956). The cochlea was perfused with 2% buffered (veronal acetate) osmium tetroxide solution and remained in the fixation fluid for 2 hours. The specimen was dehydrated in alcohol and embedded in Epon.

1  $\mu$ m thick sections were taken for light microscopy after staining with toluidine blue and thin sections were prepared for electron microscopy (stained with uranyl acetate and lead citrate).

The quantitative analysis of the sensory cells in the organ of Corti was performed by phase contrast examination of surface preparation counting present and absent sensory cells, a technique described by Kohonen,

65, Ernstsson, 1972, Ylikoski et al., 1973, Anniko, 1976.

## RESULTS

### Clinical Findings

The clinical signs were divided into those following acute (10–25 mg/kg b.w. on each occasion) or subacute intoxication (7.5 mg/kg b.w. on each occasion) and those caused by chronic intoxication (2.5–5.0 mg/kg b.w. daily for several weeks).

The former group showed general weakness as the main symptom, often combined with a loss of Preyer's reflex shortly after the injection. The weight loss was minimal, less than 5% of the initial b.w. Neurological signs did

not appear. The animals never showed a disturbance of the balance or of the righting reflex.

The chronically treated group of guinea pigs showed great changes in their body weight. Sometimes the weight decrease reached 45% of the initial weight. However, an individual tolerance to mercury chloride treatment was obvious, and some animals increased in weight during this period of time.

Many guinea pigs lost their Preyer's reflex, which, however, sometimes returned after a few days or a week. Crossing of the hindlimbs or ataxia was not observed.

### Morphological Investigation

#### 1 The cytocochleogram

**Normal distribution of cochlear hair cells in the 4th coil.** Missing outer hair cells (OHC) and an irregularity of the outer hair cell row may occur in the organ of Corti in the 4th coil, especially within 0.5 mm from the coil apex. A complete cytocochleogram of the coil was made in the control group of guinea pigs. Among the three rows of outer hair cells at 18.5–19.0 mm from the round window, the third row of sensory cells showed 5–10% loss, the second and the innermost hair rows showed about a 15–20% loss. Sensory hair bundles of the inner hair cells (IHC) were missing in about 20% of the sensory cells.

**Fig 1** Interference contrast microscopy. Chronic mercury chloride intoxication. Degenerated outer hair cells (OHC) in the second (OHC II) and the third (OHC III) rows are replaced by phalangeal scarring. Inner hair cells (IHC) are completely preserved.

**Fig 2** Light microscopy (LM). Chronic poisoning. Degeneration of outer hair cells in the second and third rows (arrows).

**Fig 3** Electron microscopy (EM). Damage to hair cell mitochondria. (A) Chronic intoxication. Increased electron density of the mitochondrial matrix and cristae integration. (B) Acute intoxication. Hemolysis of the mitochondrial membrane. Small intramitochondrial inclusion bodies adjacent to the cristae are frequently observed (arrows).

**Fig 4** EM. Chronic mercury chloride intoxication. Degenerating nerve ending of the myelinated type I outer hair cell (OHC).



20  $\mu$ m



50  $\mu$ m



0.5  $\mu$ m



1  $\mu$ m

1 day to 1 month. The control group of animals consisted of 9 young untreated guinea pigs.

The animals were weighed daily and examined for general appearance and activity. The gross neurologic investigation included gait disturbance, hindlimb crossing phenomenon and the presence/absence of Preyer's reflex.

### Morphological procedures

The cochlea was investigated by light and electron microscopy for both qualitative and quantitative analysis of cochlear damage. Specimens were taken according to the technique of Wersall (1956). The cochlea was perfused with 2% buffered (veronal acetate) osmium tetroxide solution and remained in the fixation fluid for 2 hours. The specimen was dehydrated in alcohol and embedded in Epon.

1  $\mu$ m thick sections were taken for light microscopy after staining with toluidine blue and thin sections were prepared for electron microscopy (stained with uranyl acetate and lead citrate).

The quantitative analysis of the sensory cells in the organ of Corti was performed by phase contrast examination of surface preparation counting present and absent sensory cells, a technique described by Kohonen, 1965, Ernstsson, 1972, Ylikoski et al., 1973, Anniko, 1976.

## RESULTS

### Clinical Findings

The clinical signs were divided into those following acute (10–25 mg/kg b.w. on each occasion) or subacute intoxication (7.5 mg/kg b.w. on each occasion) and those caused by chronic intoxication (2.5–5.0 mg/kg b.w. daily for several weeks).

The former group showed general weakness as the main symptom, often combined with a loss of Preyer's reflex shortly after the injection. The weight loss was minimal, less than 5% of the initial b.w. Neurological signs did

not appear. The animals never showed any disturbance of the balance or of the righting reflex.

The chronically treated group of guinea pigs showed great changes in their body weight. Sometimes the weight decrease reached 45% of the initial weight. However, individual tolerance to mercury chloride treatment was obvious, and some animals were able to maintain the same weight for several weeks.

Crossing of the hindlimbs or ataxia was not observed.

### Morphological Investigation

#### 1 The cytocochleogram

**Normal distribution of cochlear hair cells in the 4th coil.** Missing outer hair cells (OHC) and an irregularity of the outer hair cell row may occur in the organ of Corti in the 4th coil, especially within 0.5 mm from the cochlear apex. A complete cytocochleogram of the 4th coil was made in the control group of guinea pigs. Among the three rows of outer hair cells at 18.5–19.0 mm from the round window, the third row of sensory cells showed 5–10% loss, the second and the innermost hair cell rows showed about 15–20% loss. Sensory hair bundles of the inner hair cells (IHC) were missing in about 20% of the sensory cells.

**Chronic mercury chloride intoxication.** The cytocochleogram of the 4th coil in chronically treated guinea pigs showed a complete loss of outer hair cells in the second and third rows (arrows) (Fig. 2). The innermost row of outer hair cells (OHC) are completely preserved (Fig. 2). Light microscopy (LM) showed a chronic poisoning of the organ of Corti with a complete loss of outer hair cells in the second and third rows (arrows) (Fig. 3). Electron microscopy (EM) showed damage to the mitochondria (A). Chronic intoxication increased the electron density of the mitochondrial matrix and cristae integration (B). Acute intoxication showed herniation of the mitochondrial membrane. Small intramitochondrial inclusion bodies adjacent to the cristae are frequently observed (arrows) (Fig. 4). EM showed chronic mercury chloride intoxication with a complete loss of the myelin figure type in the outer hair cell (OHC).

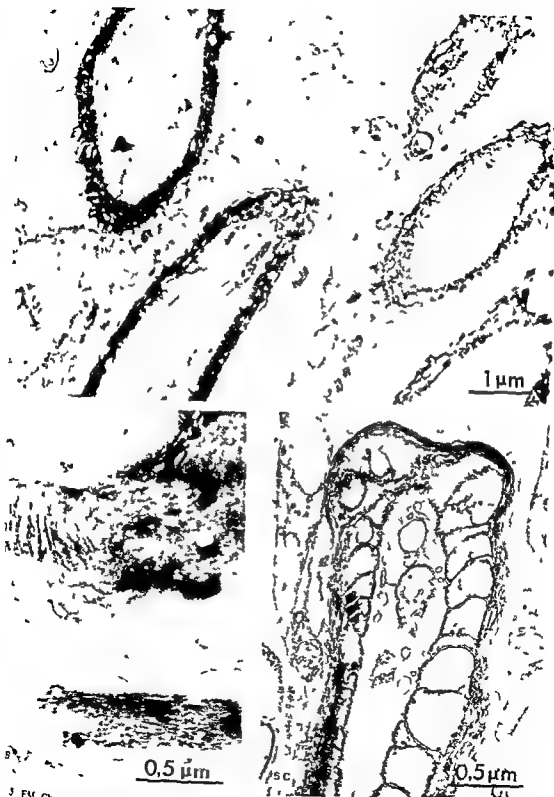


Fig. 3. EM Chronic intoxication. (A) Myelinated nerve. The axoplasm is lost and the myelin sheaths are

disintegrating. (B, C) Details from degenerating myelin sheaths showing dissolution of the myelin sheaths.

outer and inner hair cells showed a vesicular degeneration indicating a final, unspecific, common path of cell disintegration.

Concerning the morphological changes, no difference occurred between afferent and efferent nerve terminals following acute intoxication, thus indicating that both types of neural tissue were affected at the same time. If nerve endings appeared morphologically altered they were initially more affected than the adjacent hair cell although some cytoplasmic changes also occurred in the hair cell. Ultrastructural changes of nerve endings did not occur in all investigated specimens, thus indicating a great variation in the susceptibility to mercury chloride poisoning.

Degenerated nerve endings of the myelin figure type were observed below the hair cells following chronic intoxication, while the adjacent hair cell appeared ultrastructurally normal or had only minimal signs of cytological damage (Fig. 4). Adjacent nerve endings were mostly undamaged, which may be explained by the long time elapsing from the last injection until the sacrifice of the animal, indicating that damaged nerve terminals already had been degenerated. In the disintegrating efferent nerve endings the transmitter vesicles are preserved even when the mitochondria within the nerve endings seemed greatly damaged.

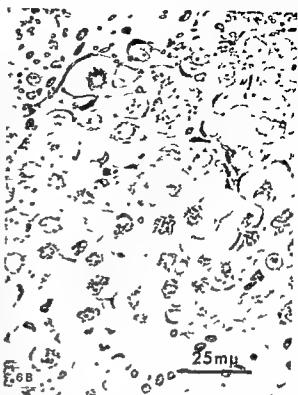
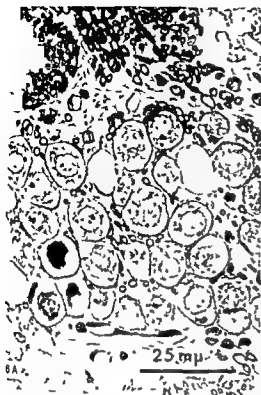
Morphological signs of demyelination appeared in myelinated cochlear nerves following chronic mercury intoxication. However, not all myelinated nerves were affected simultaneously, so many of them still appeared ultrastructurally rather undamaged while others were severely degenerating. In the disintegrating nerves the normal compact sheath of myelin layers, tightly compressed together so the extracellular spaces between the layers are completely obliterated (multilayers of Schwann cell membrane) showed vesicular dissolution by an organized arrangement of equal-sized spheres (Fig. 5C) derived from the myelin lamellae of a damaged myelin sheath (intramyelinic split) but it could also present

organelles) but in many degenerating efferent nerves foci of floccular dense bodies were observed. The cochlear ganglion cells were mostly preserved though scattered losses of ganglion cells appeared (Fig. 6A). Acute intoxication primarily affected the cochlear ganglion and sometimes caused a rapid disintegration of ganglion cells (Fig. 6B).

In most investigated specimens the stria vasculans appeared morphologically normal. However, an increased vacuolization of marginal cells could sometimes be observed following both chronic and acute poisoning (Fig. 9). In the evolution of damage to the stria vasculans, which was found mainly following chronic poisoning, an intercellular oedema in the intermediate cell layer occurred (Fig. 10) but without affecting the basal cells. Intercellular oedema can reach a considerable size but no epithelial cells were rejected from the surface of the stria vasculans or were found floating above it. The mitochondrial changes became primarily apparent in the intermediate (chromophobe) cells and the marginal (chromophil) cell extensions. The morphological transformation of their ultrastructure following the two main types of experimental conditions was similar to that described concerning mitochondria in cochlear cells (Fig. 7A, 7B).

At the same level in the cochlea as the ultrastructurally altered stria vasculans the sensory cells of the organ of Corti were still present but appeared often distorted with an increase

*Fig. 6 LM Cochlear ganglion cells. (A) Chronic intoxication. Most ganglion cells are preserved and scattered losses of cells appear. (B) Acute intoxication. General disintegration of the ganglion cells.*  
*Fig. 7 EM Morphological transformation of mitochondria in marginal and intermediate cell extensions. (A) Acute intoxication. Swelling of mitochondria with disintegration. (B) Chronic poisoning. Distinct increase in electron density of the mitochondria so that the cristae cannot be identified.*





density of the cytoplasm and some degenerating nerve endings below them. However, morphologically similar hair cell changes were also observed without corresponding alterations of the stria vascularis. The supporting tissues of the organ of Corti appeared unaffected by the mercurial poisoning.

## DISCUSSION

Recent findings of high levels of mercury in the environment have raised questions concerning its effects, and the site and mode of cellular action. The clinical features of mercurial intoxication are well known as the Hunter Russell syndrome, and the pathological findings at autopsies of such cases showed that the neurological disturbance when investigated a long time after the intoxication was attributable to the destruction of neurons in the central nervous system (Hunter & Russell, 1954; Takeuchi et al., 1962; Oyake et al., 1966).

Neuro-otological studies of intoxication by mercury have shown that the hearing impairment in the early or middle stage of the intoxication may be caused by cochlear lesions but at a later stage mainly result from retrocochlear lesions (Mizukoshi et al., 1975). Kurland et al. (1960) reported from the Minamata disease that hearing impairment (sometimes even a total loss) was an early and frequent finding following mercury poisoning (observed in more than 80% of all patients).

In the present study the degree of hearing was estimated by means of Preyer's reflex, which rapidly disappeared following acute intoxication. This was, after ultrastructural investigation of the specimens, interpreted mainly as an effect on the neural structures. The sensory cells were surprisingly well preserved although sometimes minor cytological damage occurred while the adjacent nerve endings (both afferent and efferent) were more seriously affected. In the initial stage of intoxication, these changes are likely to be reversible. Preyer's reflex often returned and when the animals were later investigated ultra-

structurally the damage was less pronounced than when studied immediately after the intoxication. In chronic mercury poisoning Preyer's reflex did not disappear until late during the treatment and this was likely due not to neural damage alone, as degenerating hair cells occurred more frequently than following acute poisoning.

Reports have been published indicating that the inner ear might have a delayed capacity to eliminate certain toxic substances thereby exposing the cochlea to a high concentration during a long period (Stupp, 1970; Stupp et al., 1973; Anniko & Plantin, 1977). Falk et al. (1974) revealed that there is a slow, continuous increase in mercury concentration in blood during chronic intoxication. Thus exposure of the inner ear to mercury would increase.

Toxic effects following metallic and organic mercury poisoning are produced by oxidation of the elemental mercury to mercuric ions in vivo (Kubasik & Volosin, 1971). Ulfvarsson (1969) reported that the distribution of mercury among the organs was dependent of the mode of administration.

Degeneration of myelinated nerves is a late mercury effect while the damage to nerve terminals occurred early during the intoxication. The early predilection for the peripheral nervous system is consistent also with other reports (Miyakawa et al., 1970, 1971). Abnormal myelin sheaths have also been shown by Homan et al. (1973) in their ultrastructural study of mercury induced primary neuropathy in rat.

Mitochondria in hair cells and nerve terminals were the first structures to become affected. This indicates also changes in the

*Fig 8* EM Chronic intoxication. Stria vascularis. Sacular degeneration of a marginal cell extension adjacent to a strial blood vessel (BV).

*Fig 9* EM Acute intoxication. Stria vascularis. Increased vacuolization of marginal cells (MC).

*Fig 10* EM Chronic intoxication. Intercellular cleft of the stria vascularis. The basal cells (BC) appear affected. MC marginal cells.



functional state. Swelling of the mitochondria is regarded to be an early sign of degeneration caused by a change in the ion balance across the outer mitochondrial membrane. Swelling is often a reversible state and has been observed in mitochondria after treatment with various drugs (Kanda & Igarashi, 1969). Southard et al (1974) reported that mercurials and other heavy metals at low concentrations profoundly affect the control of the mitochondrial function. Oxidative phosphorylation, calcium transport and phosphate accumulation are suppressed, whereas ATP hydrolysis,  $K^+$  transport,  $Mg^{2+}$  transport and mitochondrial configurational change are induced. As mitochondrial respiration diminishes, there is a simultaneous loss of cristae mitochondriales and a change in membrane permeability producing swelling is a common feature (Howell et al, 1971; Hwang et al, 1974). The reason for rupture or herniation of mitochondria is difficult to state. It may be a consequence of the swelling process or due to an increased susceptibility of the membrane following exposure to mercury to the fixation procedure, thus producing these herniations (Bagger-Sjoberg & Wersall, 1977).

The appearance of mitochondria with an extremely dense amorphous matrix without visible cristae following chronic mercury chloride intoxication may be an effect of a prolonged compensated leakage of ions due to a mercury induced disturbed non-lethal membrane permeability but a direct degenerative transformation of mitochondrial lipoproteins cannot be excluded. The inhibition of cell respiration and ATP synthesis by  $Hg^{2+}$  suggest a possible explanation of the toxicological properties.

The effect of chronic mercury poisoning in the inner ear of the guinea pig has been examined histochemically by v. Westernhagen (1969) who reported metabolic disturbances in both the sensory cells of the organ of Corti and the stria vascularis, without morphological changes. He concluded that hearing disorders following mercury intoxication are at

least in part, likely to be caused by the blockage of important metabolic pathways before other signs of cellular damage appear.

Nakazawa et al (1975) observed that the effect of mercury compounds was due to an inhibition of DNA synthesis thereby damaging the machinery of macromolecular synthesis. Similar results were obtained by Li & Trach (1974).

In the location of its damaging effect mercury is similar to atoxyl causing degeneration of the sensory epithelium of the apical coil (Anniko, 1976) in contrast to most other known causes of cochlear hair cell damage (Ylikoski et al, 1974). There is however a discrepancy between the location of the present pathological findings to the apical part of the cochlea and the high frequency deafness often described in mercury intoxication in humans (Falk et al, 1974). Damage to the guinea pig cochlea in the 3rd and 4th coils is likely to produce a low frequency hearing loss below 2000 Hz (Ylikoski et al, 1973).

The cochlea may early be damaged during mercury intoxication. Hearing impairment will therefore not only depend on the mercury effects on the central neuronal pathways but also be related to morphological changes in the inner ear.

## ACKNOWLEDGEMENT

The authors wish to thank Professor Jan Wersäll, Department of Otolaryngology, Karolinska sjukhuset, for his interest in this work and for his critical revision of the manuscript.

fully acknowledged

## ZUSAMMENFASSUNG

Die ersten und größten Veränderungen der sensorischen Epithel zeigten sich in dem apikalen Teil der Cochlea während der basalen Teil nur selten beschädigt war (Cochleogramm Studien). Die ultrastrukturelle Untersuchung erwies, daß sowohl die sensorische wie die stria vasculäre Epithel durch Merkurchlorid beschädigt werden können. Akute Intoxikation griff meist die Ne-

## REFERENCES

- afferente sowie efferente Nervterminale) an wö-  
chronische Vergiftung auch andere Strukturen  
Cochlea beschädigt
- ko M 1976 The cytochrome c oxidase in atoxyl treated  
guinea pigs *Acta Otolaryngol* (Stockh) 82 70
- iko M & Plantin L O 1977 The delayed elimina-  
tion of the ototoxic compound atoxyl from the inner  
ear *Arch Oto rhinolaryngol* 215 81
- gger Spöck D & Wersall J 1977 Gentamicin in-  
duced mitochondrial changes in the sensory cells of the  
basilar papilla in the lizard *Calotes Versicolor* *J Ultra-  
struct Res* In press
- son S 1972 Cochlear physiology and hair cell  
degeneration in experimental mercury poisoning  
*Acta Neuropathol* 17 6
- Pathol 97 297
- L 1971 Methyl mercury in fish A toxicologic  
epidemiologic evaluation of risks *Nord Hyg Tidskr*  
1 364
- R Ohno Y & Ohtake K 1971 Hearing  
disturbance in chronic intoxication with organic mer-  
cury *Audiology* (Japan) 14 484
- erman S P Klein R Talley F A & Krugman  
M R 1973 An ultrastructural study of methyl  
mercury induced primary sensory neuropathy in the  
rat *Lab Invest* 28 104
- owell N Zuiches C A & Munkres K D 1971  
Mitochondrial biogenesis in *Neurospora Crana* An  
ultrastructural and biochemical investigation of the  
effects of anoxia and chloramphenicol inhibition  
*J Cell Biol* 50 721
- unter D Bomford R R & Russel D S 1940 Poison-  
ing by methyl mercury compounds *Quart J Med* 9  
193
- unter D & Russel D S 1954 Focal cerebral and  
cerebellar atrophy in a human subject due to organic  
mercury compounds *J Neurol Neurosurg Psychiat* 17  
235
- ang K M Yang L C Camco C K Schultz  
R A Schenkman J H & Sartorelli A C 1974  
Production of membrane whorls in rat liver by some  
inhibitors of protein synthesis *J Cell Biol* 62 20
- lu M A & Abbasi A H 1961 Poisoning by ethyl  
mercury toluene sulphonamide *Br J Ind Med* 18  
303
- T & Igarashi M 1969 Ultra structural changes  
in vestibular sensory end organs after Viomycin sul-  
fate intoxication *Acta Otolaryngol* (Stockh) 68 474
- A 1965 Effect of some ototoxic drugs upon  
the pattern and innervation of cochlear sensory cells  
in the guinea pig *Acta Otolaryngol* (Stockh) Suppl  
268
- N P & Volosin M T 1973 Heavy metal  
poisoning clinical aspects and laboratory analysis  
*Am J Med Technol* 39 443
- Kurland L T Faro S N & Siedler H 1960 Mini-  
mata disease the outbreak of a neurologic disorder in  
Minamata Japan and its relationship to the ingestion  
of seafood contaminated by mercuric compounds  
*World Neurol* 1 370
- Li M F & Traxler G S 1974 Effects of mercuric  
chloride on cellular morphology and acid phos-  
phatase of tissue culture cells cultivated in suspension  
*Environ Physiol Biochem* 4 263
- Lofroth G 1969 Methylmercury—A Review of Health  
Hazards and side Effects Associated With the Emis-  
sion of Mercury Compounds into Natural Systems  
Ecological Research Committee Swedish Natural  
Science Research Council Stockholm
- Miyakawa T Deslimary M & Sumizoski S 1970  
Experimental organic mercury poisoning—pathologi-  
cal changes in peripheral nerves *Acta Neuropathol*  
15 45
- 1971 Experimental organic mercury poisoning—  
regeneration of peripheral nerves *Acta Neuropathol*  
17 6
- Mizukoshi K Nagaba M Ohno Y Ishikawa K  
Aoyagi M Watanabe Y Kato I & Ino H 1975  
Neurological studies upon intoxication by organic  
mercury compounds *ORL* 37 74
- Nakazawa N Mahuno F & Okada S 1975 Acute ef-  
fects of mercuric compounds on cultured mammalian  
cells *Biochem Pharmacol* 24 489
- Nosaka Y Setoguti A Shiga A Ohgi H Asano S  
Kiyofuji T & Togashi N 1970 Auditory vestibular  
gustatory and speech disturbances in Minamata dis-  
ease *Jap J Otol* (Tokyo) 73 1006
- Oyake Y Tanaka M Kubo M & Titibu M 1966  
Neuropathological studies on mercury intoxication—  
with special reference to distribution of mercury gran-  
ules *Adv Neurol Sci* (Tokyo) 10 744
- Rosen C-G Ackefors E & Nilsson H 1966 Orga-  
niska kvicksilverföroreningar—synpunkter på ökon-  
omiskt behov och hälsosaker *Svensk Kemisk Tid-  
skrift* 78 8
- Southard J Nitsewojo P & Green E E 1974 Mer-  
curial toxicity and the perturbation of the mitochon-  
drial control system *Fed Proc* 33(10) 2147
- Stupp H 1970 Untersuchungen der Antibiotikaspiegel  
in den Innenohrflüssigkeiten und ihre Bedeutung für  
die spezifische Ototoxizität der Aminoglykosidantibio-  
tika *Acta Otolaryngol* (Stockh) Suppl 262
- Stupp H Kupper K Lagler F Gous H & Quante  
M 1973 Inner ear concentration and ototoxicity of  
different antibiotics in local and systemic application  
*Audiology* 12 350
- Takeuchi T Monkawa N Matsumoto H & Shirashi  
Y 1962 A pathological study of Minamata disease in  
Japan *Acta Neuropath* 2 40
- Takeuchi T 1970 Biological reactions and pathological  
changes of human beings and animals under the con-  
dition of organic mercury contamination Lecture  
International Conference on Environmental Mercury  
Contamination Ann Arbor Michigan USA
- Troen P Kaufman S A & Katz K H 1951 Mer-  
curic bichloride poisoning *New Engl J Med* 244 (13)  
459

- Ulfvarson, U 1969 The absorption and distribution of mercury in rat fed organs from rats injected with various mercury compounds *Toxic Appl Pharmacol* 15 525
- Wersall, J 1956 Studies on the structure and innervation of the cristae ampullares in the guinea pig *Acta Otolaryngol* (Stockh), Suppl 126
- von Westernhagen H 1969 Innenohrveränderungen am Meerschweinchen nach chronischer Quecksilbervergiftung—eine histochemische Studie *Arch Klin Exp Ohren Nasen Kehlkopfheilkd* 193 70
- Ylikoski, J , in cooperation with Jan Wersall and Birgitta Björkroth 1974 Correlative Studies on the Cochlear Pathology and Hearing Loss in Guinea Pigs Intoxication with Ototoxic Antibiotics *Acta Otolaryngol* (Stockh) Suppl 326 1
- Ylikoski J , Wersall J & Björkroth H 1973 Hearing loss and cochlear pathology in gentamicin intoxicated guinea pigs *Acta Path Microbiol Scand Secton B* Suppl 241 30

M Anniko MD  
Dep of Otolaryngology  
Karolinska sjukhuset  
S 10401 Stockholm 60  
Sweden

## HEARING IN POP MUSICIANS

A. Axelsson and F. Lindgren

*From the Department of Audiology, Sahlgrenska sjukhuset, University of Gothenburg, Sweden*

(Received February 20, 1977)

**Abstract.** Comparatively few previous studies have investigated the hearing of pop musicians. On the average, a surprisingly low percentage (5%) of hearing loss was demonstrated in 160 pop musicians. The present material comprises 83 musicians with an average exposure of 9 years. Group mean hearing thresholds show only a slight deviation from normal. Age, weekly and total exposure may appear to increase the risk of hearing impairment. In the individual analysis, 13-30% were found to have a sensorineural hearing loss depending upon the definition of hearing loss. Subjects with hearing loss show a discrete impairment in the frequency range 3-8 kHz, considering the sound levels and the length of exposure. The incidence of hearing loss is surprisingly low. Possible reasons for this are discussed.

What the younger generation considers to be their legitimate right is often in pronounced opposition to the morals or tastes of the older generation. Nowadays, the performance of dance music constitutes such a problem. The younger generation wants to hear their pop music electronically amplified to very high sound levels, both when dancing and at concerts. This not only irritates the older generation who are quite often subjected to "noise pollution" at home or at work, but also awakens the parents' sincere concern for the hearing of their children. One of the reasons to suspect that high volume pop music leads to a sensorineural hearing loss (SNHL) is the subjective tinnitus and temporary threshold shifts (TTS) experienced by musicians and their audiences after exposure to one or more of their performances. The relationship between a temporary and a permanent hearing

loss, however, has not been conclusively elucidated. Nevertheless, it is quite apparent that those performing the music are those who are probably most heavily exposed to the high sound levels. Consequently, pop musicians should constitute the group where SNHL should be found, if pop music does, in fact, cause such hearing loss. In relation to the substantial number of general publications centering around these questions, surprisingly few investigations have concerned themselves with possible permanent hearing loss in pop musicians. The hitherto published investigations analysing hearing individually are presented in Table I. A compilation of these cases shows that a permanent hearing loss was found in 5% of the pop musicians, irrespective of exposure time, age, etc. In contrast, using group mean analysis of hearing, Fearn (1973) demonstrated a statistically significant SNHL in the frequency range 1-6 kHz in attenders at pop music performances when compared with non attenders, 14-33 dB. Attenders showed increased hearing loss with increased attendance but also with exposure to gunfire. Fletcher (1973) showed a sensorineural hearing loss at very high frequencies ( $\geq 8$  kHz) in young pop musicians, 18-21 years of age. Reddell & Lebo (1972), also adopting group mean analysis of hearing, showed a sensorineural dip at 6 kHz in 43 pop musicians with a mean age of 22 years.

The aim of the present investigation was to examine the hearing in pop musicians.

Table I Pop musicians Frequency of permanent hearing loss (PHL) caused by pop music

Author	Number examined	Number PHL
Fluur (1967)	13	0
Rintelmann & Borus (1968)	42	2
Ewertsen (1971)	26	0
Jatho & Hellman (1972)	65	5
Chuden & Strauss (1974)	14	1
	160	8 (5%)

## MATERIAL AND METHOD

By advertisements in the news media and through personal contacts, pop musicians were asked to attend the examination. Tested musicians were asked to encourage their colleagues of the band and other musicians they knew to take part in the examination. In many cases it was possible to examine all members of a pop band at a training session or concert. In these instances, hearing was tested in a mobile van. A total of 69 musicians, 4 disc jockeys, 4 managers and 6 sound engineers were included in the audiological examination. Of the total 83 tested, 52 constituted all the members from 8 pop bands, while the rest were members of many different pop bands. This material includes 18 musicians from three internationally well known English pop bands. The individual distribution with respect to the type of musical instruments played is presented below.

guitar	26
base	8
piano or electric organ	12
drums	16
saxophone	4
singers	4
violin	1
more than one instrument	
managers	
sound engineers etc	12
	83

The average age of the group was 26.5 years (17–40 years). The average exposure time for the musicians was 9.3 years; they performed 18.3 hours (average) a week; 3 hours each session.

Pure tone air conduction thresholds were obtained at both ears for all musicians. When the thresholds exceeded 20 dB, bone conduction testing was also carried out. Pure tone audiometry was performed in a sound proof booth. The audiometers used were Madsen TBN-80, Madsen OB 70 and Kamplex. All data were computer analysed.

Statistical treatment was performed according to Z-test, using a significance level of 0.05.

Hearing loss is here defined as a mean threshold level greater than 20 dB at one of the frequencies 3–8 kHz at one ear.

## RESULTS

### Group means

The average hearing in pop musicians is presented in Fig. 1. It can be seen that there is a slight difference between the ears, in that the right ear is better in the low frequencies and the left ear is better in the high frequencies. This is due exclusively to a marked difference between right and left ears in younger musicians. It can also be seen that thresholds are most severely impaired at 6 kHz, with an average of 20.4 dB at the right ear and 17.1 dB at the left ear.

The material was analysed in many ways in order to establish parameters contributing to possible SNHL. The results are presented elsewhere (Axelsson & Lindgren 1977).

In summary, the following parameters:

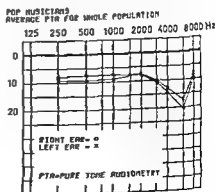
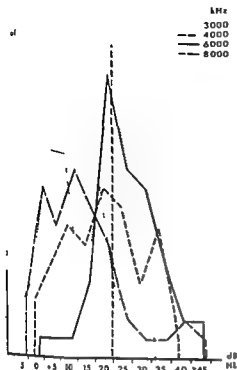


Fig. 1 Average pure tone audiogram



2 Histogram showing pure tone loss in relation to frequency

ear to have a negative influence on hearing in pop musicians

older age

long exposure time

long weekly exposure

long exposure per session

playing drums

participation in military service

listening with stereophonic headphones

#### Individual analysis

In the individual analysis each ear with a pure threshold exceeding 20 dB at any frequency from 3 to 8 kHz was taken into account. It was found that 38 musicians had a SNHL. The right ear only was affected in 11 cases, the left only in 5 cases, and both ears in 22 cases. A total of 13 cases were excluded as their hearing loss was reasonably explained by causes other than pop music. These causes were

congenital loss	1 case
age (according to Spoor 1967)	5 cases
SNHL, unknown etiology verified before exposure to pop music	3 cases
Occupational hearing loss (other than from pop music)	4 cases
Total	13 cases

After exclusion of the 13 cases 25 pop musicians remain with SNHL, i.e. thresholds exceeding 20 dB at least at one frequency from 3–8 kHz at one ear.

Contrarily, certain other possible causes of SNHL were not regarded as sufficient to exclude the patients from the material. These were

military service (4–19 months)	9 cases
previous otitis media	7 cases
bilateral mastoidectomy	1 case
amateur shooting	2 cases
head concussion	1 case

The distribution of the hearing loss at the high frequencies for the 25 musicians is shown in Fig. 2. It can be seen that most ears are within normal limits and that there is a considerable difference between frequencies. At 3 and 8 kHz few ears were poorer than 25 dB. The frequency 4 kHz shows a fairly equal distribution of ears above or below this level, while many pathological ears are found at 6 kHz, which is the most commonly impaired frequency. It can also be seen that very few ears exceeded a 35 dB hearing level.

To establish the incidence of SNHL, three different limits were set, as shown in Table II. It can be seen that the SNHL in pop musicians in the present material ranges between 13 and

Table II Incidence of sensorineural hearing loss

No. tested	111
No. with SNHL > 20 dB in one ear and one frequency (3–8 kHz)	38
No. with SNHL after correction for age	25 (30%)
"    "    "    "    "    "	
"    "    "    "    "    "	18 (22%)
"    "    "    "    "    "	11 (13%)
"    "    "    "    "    "	Unilateral 7 cases (8%)
"    "    "    "    "    "	bilateral 4 cases (5%)



Table III The worst cases (pure tone audiometry &gt;20 dB in one or both ears)

Subj no	No tested musicians in the band	Age	Instrument	Played years	Plays (hours/week)	Plays (hours/session)	Milit ser vice (mo)	Otitis media	Head con cus media tion	Mean hearing loss (in dB 3 4 6 8 kHz)		Max hearing loss in dB (frequency kHz)	
										Right	Left	Right	Left
1		28	Guitar	8	50	2	4	+		24	11	45 (6)	20 (6)
2		40	bass	18	18	3	12			26	30	40 (8)	55 (8)
3	5	35	Guitar	16	25	4	-	+		25	13	35 (4)	25 (4)
4	7 Same band	32	Piano organ	10	10	1	10			21	23	25 (7.3)	30 (4)
5	7 Same band	35	trombone	20	10	1	19		+	23	25	30 (4)	25 (6)
6	6	28	Singer	18	30	4	13			11	25	20 (4.6)	40 (4)
7	5	27	Bass guitar	5	70	1.5	-			14	29	20 (4)	40 (8)
8	6 Same band	27	Stage monitorist	9	30	2	-			29	31	40 (6)	35 (4)
9	6 Same band	28	Bass	15	50	2	-	+		18	23	30 (6)	35 (4)
10	6 Same band	29	Drums	10	20	2	-	+		20	24	25 (6)	30 (6)
11	6 Same band	28	Singer	15	50	2	-	+		21	30	35 (4)	30 (5)
Total average		30.6		13	33	2.2							
Total material average		26.5		9.3	18	3							

30%, depending upon the definition of hearing loss. In most cases the SNHL was discrete. The 11 worst cases of SNHL in pop musicians are presented in Table III. The cases had an average loss from 3-8 kHz greater than 20 dB. It can be seen that these cases were, on average, 4 years older than the group as a whole. In addition they had played an average of 13 years, compared with the overall average of 9.3 years. The weekly exposure for these musicians was 33 hours and they had played an average of 2.2 hours/session, the means for the whole group were 18 hours and 3 hours, respectively. The table also shows the average hearing in the high frequencies and this again confirms that the hearing loss was, in general, small.

## DISCUSSION

Occupational noise is often considered more or less "necessary" and difficult to avoid. In contrast, noise during leisure activities seems more "unnecessary" and easier to avoid. It

also appears particularly important for the exposed to occupational noise to avoid no leisure activities, in recent years a new, no leisure activity has been introduced in society, i.e. amplified pop music. As a sign of the continuous protest of the young generation toward "old folks" or for other unknown reasons this pop music is presented at high sound levels, clearly above the hearing damage risk level. Occasional sound level measurements as well as noise dosimetry have been used as "proof" of the expected noxious effect of this music on hearing. Screening it have shown more young individuals to have hearing loss, which has also been blamed on pop music (Lipscomb, 1969a). However, amazingly few investigations have reported the individual hearing in those obviously exposed to the pop music, i.e. pop musicians. Those studies that have been done have demonstrated a surprisingly low incidence of SNHL (Table I).

The following factors have been considered as evidence of the harmful effect of pop music on hearing:

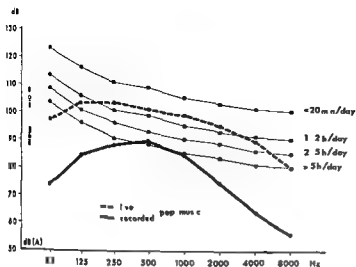


Fig 3 Live and recorded pop music in relation to the Swedish damage risk criteria

ry high sound levels  
gh equivalent sound levels  
TS after exposure  
earing screening showing young individuals  
with hearing loss  
idely exceeded established damage risk cri-  
teria

mpared hearing in pop music attenders in  
comparison with non attenders in hearing  
tests (Fearn, 1973)  
air cell damage in inner ears subjected to pop  
music, found at histological examination  
(Lipscomb, 1969b; Bohne et al., 1976)

How is it then possible that previous studies  
found only 5% of pop musicians to have  
NHL and the present investigation found  
1 or 30%, depending upon where the limits  
or normal hearing are set? Certain factors  
tend to explain such a low occurrence of  
hearing loss. The dominant frequencies of  
pop music are low, the 250–500 Hz range is  
maximally amplified. Low-frequency noise is  
less damaging to the inner ear, perhaps  
through the protective action of the stapedius  
reflex. The presentation of loud pop music is  
often interrupted by pauses which offer at  
least some possibility of recovery and rest.  
The exposure time is very different from that  
in industrial noise exposure. For listeners

this would probably amount to 2 nights/week  
(6–8 hours) and for pop musicians probably  
2–3 times that, such as the 18 hours average  
found in the present investigation.

Lately, an increasing amount of evidence  
has accumulated demonstrating the relatively  
high risk of hearing damage from impulse  
noise in relation to other types of noise (Bruehl,  
1976). Pop music is presented amplified over  
loudspeakers and is characteristically a fluc-  
tuant noise with little inclusion of impulses.  
These factors tend to make the picture of hear-  
ing loss in connection with pop music some-  
what less disastrous. Two other possibilities to  
explain the low incidence of SNHL in pop  
musicians have been suggested by Fearn  
(1973). He believes that those pop musicians  
experiencing a substantial hearing loss, either  
temporary or permanent, possibly quit playing  
earlier than their less sensitive companions. It  
is also possible that those musicians with a  
permanent hearing loss avoid taking part in  
this type of examination. On the other hand,  
reservations also have to be made that hearing  
testing was performed on only one occasion,  
which would tend to give inferior results rather  
than the reverse. Also in some instances the  
testing was performed immediately before a  
concert and there is no certainty that musi-  
cians did not have some TTS, e.g.

posure to sound tests on stage before the concert

Whittle & Robinson (1974) presented a compilation of sound levels from many different investigations. If these average sound levels are related to the Swedish noise damage risk criteria it can be seen that recorded music in discotheques on average hardly reaches hearing damage levels if exposure time is less than 5 hours (91 dB(A)), Fig. 3. In this connection it is important to emphasize the possible harmful effect of listening to pop music with stereo headphones such that much higher sound levels are incident on the drum membrane. In Fig. 3 we can also see the average octave analysis of live pop music. It can be seen that an unprotected ear could be exposed approximately 30 min/day to live pop music (105 dB(A)) without risk of hearing loss. If this figure is converted to a weekly exposure of 2.5 hours it appears that occasional patrons of discotheques could expose themselves to the live pop music once or twice a week without risk of hearing loss. It is obvious that the picture is clearly different for pop musicians. In general, pop musicians are exposed to the loud music for many more years than the audience. It is also clearly seen that pop musicians with an average exposure of 18 hours a week, as in the present investigation, far exceed the risk level. In this respect the finding of a discrete SNHL in 13–30% of pop musicians which seldom exceeded 30 dB at any frequency, is surprising. The group mean hearing, though, shows a dip at 6 kHz, very similar to that presented by Reddell & Lebo (1972) and indicative of an early slight sensorineural impairment in these fairly young individuals.

There is a well known large inter-individual difference in sensitivity to noise. Basically, we do not know what factors contribute to these inter-individual differences. We would like to make the suggestion that there may be a difference in inner ear circulation on a hormonal basis if high sound levels are experienced as "beautiful music" versus as "terrible noise".

The male ear, particularly is exposed to

many different noises from early childhood: squeaking toys, fire crackers, toy guns etc. It is obvious that in general no single factor can be blamed for an individual hearing loss but that such a loss rather is the result of repeated trauma to the inner ear. Consequently, there is seldom reason to incriminate pop music *per se* as the cause of hearing loss. Contrarily, it is important to remember that a normal audiogram or one with only a slight hearing loss does not necessarily reflect a small amount of morphological damage to the inner ear. Even with a normal pure tone audiogram sensory cell loss can be substantial. Furthermore, the presentation of pop music at the high sound levels appears senseless. If the younger generation considers high sound levels necessary for enjoyment of their music, it is our obligation to determine the high acceptable sound level for these performances. A level of 105 dB(A) is unacceptable from the audiological point of view. A level of 85 dB(A), the Swedish hearing damage risk level, is probably unacceptably low from an audience point of view. Maybe a level of approximately 95 dB(A) would satisfy both parties in being noisy enough to be enjoyable on the one hand and restrained enough to preserve normal hearing on the other.

## ACKNOWLEDGEMENT

Computer service was offered by electrical engineer Harald Nyman. The present investigation was supported by Socialstyrelsen and Stiftelsen Tysta Skolan.

## ZUSAMMENFASSUNG

Es gibt verhältnismäßig wenige Studien über das Gehör der Popmusiker. Überraschend wenige Musiker mit Hörverlust wurden gefunden. Im Durchschnitt 5% von Popmusikern. Unser Material enthält 83 Popmusiker einer durchschnittlichen Exposition von 9 Jahren. Gehör der Musiker zeigt im Durchschnitt nur einen geringen Verlust. Der Risiko eines Gehörverlustes nimmt mit Alter, Expositionszeit pro Woche und Gesamterfahrung zu. In der individuellen Analyse wurde ein Gehörverlust in 13–30% gefunden, abhängig davon wie man normales Gehör definiert. Die Popmusiker mit Gehörverlust

ine diskrete Verminderung in 3-8 kHz. Mit Hin-  
auf die Schallpegel und die Expositionszeit ist die  
anz von Gehörverlusten erstaunlich klein. Ver-  
lichkeiten dieses zu erklären werden dis-

## REFERENCES

- sson A & Lindgren F 1977 Factors increasing the  
risk for hearing loss in pop musicians. *Scand Audiol*  
6 177
- B A Ward P H & Fernández, C 1976 Irre-  
versible inner ear damage from rock music. *Trans Am*  
*Acad Ophthal Otol* 82 50
- zel M V 1976 Do we measure noise correctly? *Bruel*  
*& Kjaer Technical Review* 1 3
- iden H & Strauss P 1974 Gibt es eine Lärmschwer-  
hörigkeit bei Diskjockeys? *Mtschr Ohrenheilkd* 108  
377
- ertsen H W 1971 Beat music and damage to hearing.  
*Scand Audiol* 20 154
- R W 1973 Pop music and hearing damage. *J*  
*Sound & Vibration* 29 390
- er L 1973 High frequency hearing and noise ex-  
posure. *Proc Intern Congr on Noise as a Public*  
*Health Program*. US Environmental Protection  
Agency Washington
- Fluur E 1967 Popmusiken som bullertrauma. *Läkartid-  
ningen* 64 794
- Jahto K & Hellman H 1972 Zur Frage des Lärm und  
Klangtraumas des Orchestermusikers. *HNO* 20 21
- Lipscomb, D M 1969a High intensity sounds in the re-  
creational environment. *Clinical Pediatrics* 8 63
- 1969b Ear damage from exposure to rock and roll  
music. *Arch Otolaryngol* 90 545
- Reddell R C & Lebo C P 1972 Ototraumatic effects of  
hard rock music. *Calif Med* 11 1
- Rintelmann W F & Borus J F 1968 Noise induced  
hearing loss and rock and roll music. *Arch Otolaryngol*  
88 377
- Spoor A 1967 Presbycusis values in relation to noise-  
induced hearing loss. *International Audiology* 6 48
- Whittle L S & Robinson M W 1974 Discotheques  
and pop music as a source of noise induced hearing  
loss. *National Physical Laboratory Acoustics Report*  
AC p 66
- A Axelsson M D  
Dept of Audiology  
Sahlgrenska Sjukhuset  
S-41345 Göteborg  
Sweden

## A CLINICAL PILOT STUDY ON PREFORMED AUTOLOGOUS OSSICLES II

A Tjellstrom, J Lindstrom, T Albrektsson, P -I Brånemark and O Hallen

*From the Laboratory of Experimental Biology Department of Anatomy and the  
Department of Otolaryngology University of Göteborg Göteborg Sweden*

(Received July III 1977)

**Abstract** A research project concerning preformed autologous ossicles has been going on for more than ten years. Animal experimental studies were followed by a clinical pilot study which showed that bone could be produced in a titanium mould placed in the proximal tibia metaphysis. In the present investigation in which another five patients were operated on the experimental design was changed compared to the first pilot study and bone production was improved. In addition to the tibial moulds ten titanium cylinders were placed around a bony bridge prepared in the linea temporalis of the ear to be reconstructed. Nine out of ten of these cylinders contained bone suitable for ossiculoplasty. Histology showed higher osteocyte density and a higher proportion of vital looking osteocytes in the preformed grafts compared with a peroperatively sculptured graft of cortical bone. On histochemical investigation the preformed graft cells seemed viable at the time of transplantation. The technique for obtaining a preformed autologous ossicle in the titanium cylinders in the temporal bone was / to perform and the patients did not experience any be small. The risk of infection in this area was judged

A project concerning preformed autologous ossicles has been going on since the late nineteen sixties. In a series of animal experiments it was shown that bone tissue with specific anatomy could be produced in titanium moulds placed in the proximal tibial metaphysis. It was also shown that this bone could be produced in a suitable shape and size for use as an ossicular prosthesis. This experimental work was published in 1976 (Hallén et al., 1976).

The first clinical pilot study on preformed autologous ossicles started in 1976 and was recently reported (Tjellstrom et al. 1977).

Titanium moulds with room for two ossicles each were placed in the proximal tibial metaphysis of 5 patients with ossicular defects. When the moulds were extracted bone was found in seven of the chambers but only in two of them was the bone stable enough to be used as a prosthesis.

Based on the results of the first clinical pilot study, the next phase of the project was planned and carried out during 1976-77. The results are presented in this report.

The aims of clinical pilot study II were:

- 1 to find out whether the quality of the bone used for ossiculoplasty could be improved as compared with pilot study I. The following changes were decided upon:
  - (a) to put bone tissue into the chamber of the mould at the time of insertion
  - (b) to redesign the chambers, making the narrowest parts slightly wider
  - (c) to change the level of the mould in relation to the periosteum and the cortical endosteum,
  - (d) to shorten the time between insertion and removal of the moulds,
  - (e) to change the arrangement for fixation of the mould to the bone,

2 to find out whether preformed ossicles could be obtained more simply by implantation of a cylindrical titanium mould in the mastoid process of the ear to be reconstructed.

3 to find out whether there were any differences concerning histology and histochem-



Fig 1a The titanium mould assembled and taken apart

try between preformed ossicles and non-reformed ossicles prepared and shaped out of one taken from the mastoid process

### MATERIAL

The patients were selected from the waiting list for ear surgery at the ENT Department, Sahlgrenska Hospital, Goteborg, Sweden, according to the same criteria as in the clinical pilot study I, viz

1 age between 20 and 50,

2 known or strongly suspected ossicular damage,

3 a dry ear without discharge for at least one year,

4 no signs of osteitis or cholesteatoma,

5 a good speech discrimination score,

6 normal hearing in the contralateral ear,

7 no general disease,

8 a normal radiological examination of the proximal tibial metaphysis

Table I Preoperative situation in each of the patients (unfilled=defect)

Patient	Sex	Age	Diseased ear	Drum	Ossicular defect	Pure tone audiogram	Speech discr score	A/B gap
AA	♀	26	dx	atrophic but intact		55 dB	100%	42 dB
SK	♀	36	sin	thick but intact		43 dB	96%	28 dB
EL	♀	40	sin	normal		76 dB	64%*	55 dB
AN	♂	32	dx	normal		70 dB	76%	47 dB
HF	♂	24	sin	atrophic but intact		35 dB	96%	23 dB

\* at 95 dB

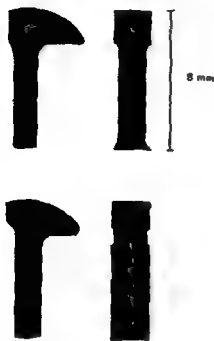


Fig 1b Shape and size of the chambers in the mould. Upper figures intended for cases in which only the foot plate was left. Lower figure intended for patients with an intact stapes.

Five patients were selected and clinical data for each patient are presented in Table I.

All the patients were thoroughly informed about the aims of the investigation and the potential risks with this type of surgery.

For the histological and histochemical investigation ten pieces of cortical bone were taken from the mastoid process of patients undergoing surgery for chronic ear disease.

## METHOD

(A) Five moulds were made out of titanium. Each mould contained two chambers, one for use if the stapes was intact and another for use if only the stapedial footplate was left in the middle ear (Fig 1). The arrangement for fixation of the mould to bone was as shown in Fig 2 and a secure arrangement was achieved with two separate titanium screws.

(B) Ten titanium cylinders were constructed. The shape and size of the two are shown in Fig 3. Each cylinder had two small grooves where a suture could be applied to adapt the pairs of cylinders snugly to each other. All inside surfaces of the moulds and cylinders were carefully polished and then cleaned and autoclaved as in the first pilot study.

## Surgical procedure 1A

The procedure described in pilot study 1 followed where applicable. Anaesthesia was given and the soft tissue was handled in

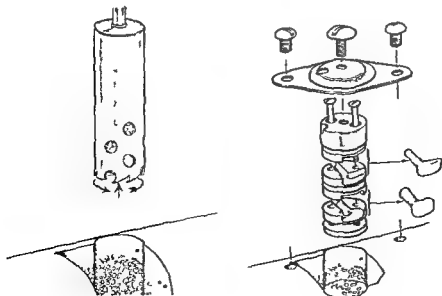


Fig 2 Extraction of bone plug and fixation arrangement of the titanium mould in tibia.



Fig 3a The titanium cylinders for the linea temporalis

Fig 3b Shape and size of the titanium cylinders

the way as in study I. At the level of the anterior tibiae a hole was made in the anterior medial surface of the tibia with a high speed burr and a cylinder of bone was taken out. From the cortical endosteal layer of the tibia a bone plug, a slim bone prosthesis was prepared with a diamond burr. The preparation was done under irrigation with the pa-

tient's own plasma. This bone prosthesis was placed in the wide chamber. Care was taken to adjust the level of the chamber close to the periosteal layer but entirely within the bony cortex. The narrow chamber was filled with spongy bone and red bone marrow, taken from the opened marrow cavity. The mould was then put in place and immobilized by the

Table II Schematic illustration of the amount of bone produced in each patient (unfilled = effect)

Patient	Chamber close to the cortical endosteum	Chamber towards the marrow cavity	Preformed bone in os temporale
AA			
SK			
EL			
AN			
HF			



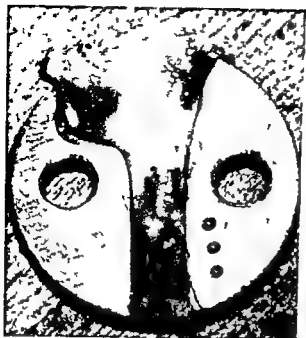


Fig 4 Preformed autologous ossicle from the tibia mould (Upper) The ossicle in the chamber half (Lower) The ossicle taken out ready to be used for the ossiculoplasty

fixation arrangement. The level of the deepest of the chambers was close to the cortical endosteal layer.

The periosteum and other soft tissue replaced. The rest of the surgical procedure and after care of the patient was the same as described in the first pilot study.

#### *Surgical procedure 1B*

This procedure was performed in the same session as procedure 1A. Under local anaesthesia with 5–10 cc of 1% Xylocain with epinephrine 1:100 000, an incision was made behind the ear with the ossicular dam. The periosteal flap was elevated and the temporalis was identified. Under penicillin saline irrigation to minimize heat trauma, bony bridges were prepared and two tit cylinder halves were adapted around the ossicle. The cylinder halves were kept in place with silk sutures placed in the groove on the outside of the cylinder. One short and one long suture were used in each patient. The penicillin flap was replaced and kept in place with catgut sutures, and the skin was sutured. A bandage was applied but the next day it was removed and only a piece of adhesive tape was left in place. The skin sutures were removed one week after surgery.

#### *Surgical procedure II*

The second operation was planned and carried out according to the following schedule: 6 months after insertion of the moulds in the cylinders, the patient was admitted to hospital for the ossiculoplasty. The reconstruction was done under general anaesthesia. The middle ear was exposed through a postauricular incision. The titanium mould was taken out from the tibia and the cylinders were exposed. On removal of the cylinders, sufficient bone was taken to permit sculpturing a head for a prosthesis. The bone that was surgically judged likely to give the best result was used for the reconstruction. All handling of the preformed bone was done in a glass vessel containing the patient's own plasma. In patients with a perforated tympanic membrane, autologous temporal fascia was used for gasketing with an underlay technique. When sur-



Fig. 5 Preformed autologous ossicle from the linea  
(Upper) One cylinder half taken away (Lower)  
The ossicle taken out ready to be inserted into the  
middle ear

drum graft was needed, Gelfoam® was used. No Gelfoam® was allowed to come into contact with the new ossicle. The ear canal was packed with rayon strips and acrylic cotton, which was removed one week after the operation.

Preformed bone from the tibia mould and the temporal cylinder of the 5 patients not included for the reconstruction were secured for histology and histochemical investigations. In all the cases it was possible to get a piece of bone from the wide tibia mould for these in-

vestigations. Cortical bone from the mastoid was taken from 2 of the patients and adjusted to the shape of an ossicular prosthesis with diamond burs under generous saline cooling. Another eight grafts of the same kind were taken from other patients and prepared in the same way in order to compare non preformed bone with preformed bone.

All specimens for histology and histochemistry were placed directly in the operation room into Histocon (Histolab, Göteborg) and were within half an hour placed in 10% EDTA of pH 6.95, +4°C (Fullmer, 1966). (The histochemical investigations were performed by Professor Guy Heyden, Department of Histopathology, University of Göteborg.) All preparations were decalcified in EDTA for ten days. After decalcification the preparations were frozen in isopentane at -140°C. The specimens were then sectioned in a cryostat at -20°C. They were stored for complementary analyses at -70°C. Preparations for histology were stained with hematoxylin eosin and van Gieson. For lipid histochemistry oil red was used. The enzyme histochemical methods included registrations of adenosine triphosphatase (Ca ATPase pH 9.4 (Magnusson & Linde, 1974), NADH<sub>2</sub> and NADPH<sub>2</sub>-diaphorase (Chayen et al., 1973), alkaline and acid phosphatase (Barka & Anderson, 1963) activities. The enzyme histochemical tests were performed in order to disclose metabolically active cells (NADH<sub>2</sub> and NADPH<sub>2</sub>-diaphorases) involved in hard tissue function (ATPase, alkaline phosphatase) and hard tissue resorption (acid phosphatase). The ATPase recordings could also be employed to show the distribution of viable blood vessels (Heyden, 1969), as defined by histochemical method.

## RESULTS

The clinical findings in each patient are presented separately below and a schematic illustration of the amount of bone found in each mould is presented in Table II.

*Patient A* A Bone was found in the

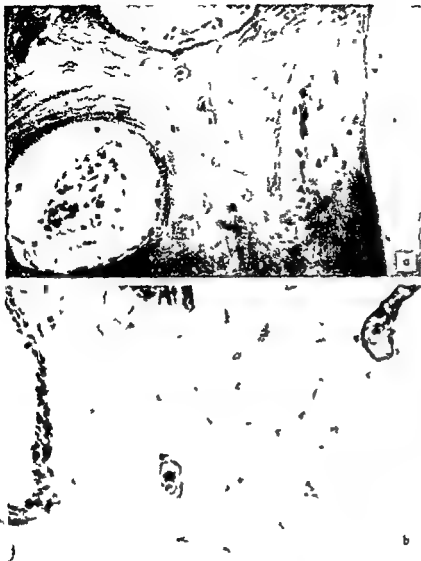


Fig 6 Preformed bone from the tibial mould of patient A. (a) The lacunae contain vital osteocytes throughout the preparation. (b) Histochemical investigation shows strong activity of NADH-peroxidase.

chambers of the mould placed in the tibia. Clinically, the bone tissue was stable enough to be used as a prosthesis (Fig 4). A thin coating of soft tissue was found around the ossicles. In the titanium cylinders placed in the linea temporalis at surgical procedure IB clinically stable bone was found and here too a coating was noted (Fig 5). This fibrous coating seemed to be thicker than that of the prosthesis obtained from the tibial mould. For the reconstruction of the ossicular defect the wide prosthesis from the tibia mould was selected and an assembly technique was used.

**Patient S. K.** The findings were as in patient A. A and the reconstruction was performed with the wide tibial prosthesis

from the stapes head to the drum (slipped prosthesis).

**Patient E. L.** The findings were as in A. A and the same type of reconstruction was performed.

**Patient A. N.** In the wide parts of the chambers of the tibia moulds bone tissue was found but the shafts were not stable and consisted only of fibrous tissue. Both the cylinder from the linea temporalis, on the other hand, contained stable bone tissue with a fibrous coating and the shorter of these was used for the reconstruction using an assembly technique.

**Patient H. F.** In the wider chamber of the tibia mould clinically stable bone tissue was



Fig 7 Preformed bone from the os temporale cylinder of patient S K (a) The lacunae contain vital osteocytes. A soft tissue layer lines the preparation (b) NADH-F activity is strong in the bone and in the soft tissue layer

and but the narrower one had only a fibrous coating and could not be used as a prosthesis. Of the bony bridges prepared in the linea temporalis at surgical procedure IB one had been resorbed at its most central part whereas the other was well retained. There was no difference as compared with the other patients; however, the fibrous coating seemed to be somewhat thicker in this case and it was not firmly attached to the bone tissue. The tibia prosthesis from the tibia was found suitable and was used for the reconstruction with the malleus-stapes assembly. All patients in this study complained of discomfort from the leg but much less than in the previous study. None of the pa-

tients complained of any discomfort from the implantation of the titanium cylinders in the linea temporalis. To diminish the risk of infection from the surgical procedures, all the patients were given Lincomycin (Lincocin®), 0.5 g twice daily for 5 days. No signs of local infection were found after any of the surgical procedures. When the patients were checked one month after surgery they all had an intact tympanic membrane and hearing tests showed that in all the air/bone gap was closed within 10 dB.

The tissue sections stained for histology on the bone from the tibia mould showed in 4 of the patients osteocyte lacunae containing vital looking cells. The enzyme histochemical m-

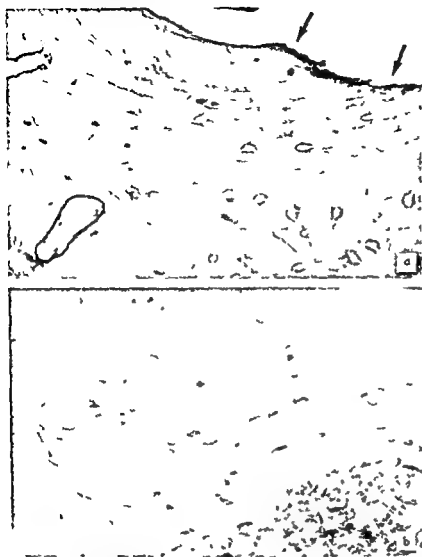


Fig 8 Not preformed bone taken from os temporale. (a) The arrows on the top right side of the picture indicate an area where the bone has been sculpted by the burr. While no osteocytes to the left (on the far side of the surgical trauma) seem vital the lacunae in the right part (under the sculpted area) are empty throughout the preparation. (b) Weak or no NADH<sub>2</sub> enzyme activity is found in the bone while the soft tissue away from the trauma shows NADH<sub>2</sub> activity.

investigations showed activity of diaphorases and ATPase indicating viability of the bone and endothelial cells (Fig 6). Strong alkaline phosphatase activity and weak or no acid phosphatase activity were found in the grafts. In the central part, separated by cement lines, there was a minor bone area with lacunae containing pyknotic nuclei. This central part showed positive lipid staining indicating degenerative processes. This bone also lacked diaphorase and ATPase activities.

In the fifth patient, H & F all the bone from the tibial mould taken for microscopic investigation showed empty lacunae, lacunae with pyknotic cells and no diaphorase enzyme activities.

The histological and histochemical investigations of the bone from the os temporale cylinder showed the same general pattern as found in the tibia mould bone of the 4 patients described above. The preformed bone from tibia and os temporale was surrounded by an enveloping sheath of connective tissue with strong enzyme activities. This connective tissue sheath was somewhat thicker around the os temporale bone (Fig 7).

The cortical bone taken from the mastoid process and sculptured preoperatively had a zone about half the width of the graft where the osteocyte lacunae were empty or contained pyknotic nuclei. The entire graft showed weak or no enzyme activity (Fig 8).

## DISCUSSION

The results obtained in clinical pilot studies I and II show that the findings in the animal experiments are also valid for man. It was thus possible to obtain a preformed autologous ossicle suitable for ossiculoplasty from a human mould placed in the proximal tibial

In study I two of the ten chambers contained of suitable quality for ossiculoplasty. In study II this proportion was improved to seven of ten. It seems reasonable to assume that the changes made in the latter study were reliable for the better result but the limited number of patients does not permit a detailed evaluation of the importance of each single factor.

A histological comparison between the bone from the tibial mould and the mastoid and a conventional, non-ossicle graft from the temporal bone was performed. The preformed bone had a higher density of osteocytes. A higher density of the osteocyte lacunae of the preformed bone contained nuclei than in the ossicle grafts sculptured peroperatively. In the chamber filled with spongy bone and marrow hematoma a marrow space surrounded by a cortical shell could be recognized. The same type of marrow cavity could not be identified in the prosthesis where a piece of cortical bone had been placed at insertion. This ought to lead to a somewhat slower revascularization and also delay the other phases of integration of the graft.

The histochemical investigation of diaphorase, ATPase and alkaline phosphatase enzyme activities showed a strong activity in the preformed grafts. This was valid for all preformed grafts with the exception of the tibial mould bone from one of the patients that showed no enzyme activity at all.

The fact that there was strong alkaline phosphatase activity and weak or no acid phosphatase activity in the preformed transplants indicated that bone formation domi-

nated over bone resorption (Magnusson et al., 1977).

The non viable small bone areas separated by cement lines found in the centre of the tibial mould preparations were probably remnants of the bone implants placed in the mould when it was installed in the tibia.

The histochemical findings indicate that the cells of the preformed grafts were viable ("active") at the time of transplantation. This is probably due to the fact that the surgical trauma (handling, vibration, heat) inevitable during a surgical procedure is much milder for a preformed graft than for a graft taken and prepared during the reconstruction of the ossicular chain. Thus, the properties which ought to make the preformed ossicle superior to that peroperatively sculptured are vital and it is covered by a fibrous lining.

The procedure for producing a preformed ossicle in a titanium mould placed in the tibia is, however, somewhat too complicated to be used as a routine method. In spite of the fact that no osteomyelitis was seen, this risk cannot be neglected and the special antiseptic arrangements put special demands on the surgical team. The discomfort from the leg that most patients experienced should also be kept in mind.

If a method is to be used in routine work it has to be simple and safe. The procedure in study IB is a step in the right direction and could easily be integrated in a staged procedure. The anatomical region is also well known to every otosurgeon. Although the number of patients used in this study was restricted it seems possible to get a high success rate with titanium cylinders in linea temporalis and the surgical technique could possibly be further refined. The next phase of this investigation will be to take advantage of the experience achieved in the pilot studies. Further pilot studies are, however, necessary and planned before this new concept in ossiculoplasty can become a clinical routine procedure. It is also important to stress that the ultimate value of this new type of prosthesis can only be

after a long period of careful follow-up in a large number of patients

## ZUSAMMENFASSUNG

Seit mehr als zehn Jahren ist ein Forschungsprojekt betreffend präformierter autologer Gehörknöchelchen im Gange. Nach Tierexperimenten folgten klinische Versuche. Diese zeigten daß Knochengewebe in einer Titaniumform, die in die proximale Tibiametaphyse eingepflanzt wurde, produziert werden konnten. In der vorliegenden Arbeit wurden noch fünf Patienten operiert. Das experimentelle Muster wurde diesmal im Vergleich zu den früheren klinischen Studien geändert, und eine größere Menge von Knochengewebe wurde erzeugt. Außer den Tibiaformen wurden zehn Titaniumzylinder um eine Knochenbrücke in Linea temporalis eingebaut. Neun von den zehn Zylindern enthielten für Ossiculoplastik geeignetes Knochengewebe. Die nachfolgende histologische Untersuchung zeigte lebendiges Knochengewebe mit ausgefüllten Osteozytenlakunen. Zur Zeit der Transplantation zeigten sich die Transplantatzellen bei der histochemischen Untersuchung lebendig. Die Implantation der Titaniumzylinder im Schläfenbein war einfach, und belastigte die Patienten wenig. Die Infektionsgefahr in diesem Gebiet konnte fast vernachlässigt werden.

## REFERENCES

- Barka, T. & Anderson, P. J. 1963 *Histochemistry* Hays & Row, New York
- Chayen, J., Bitensky, L. & Butcher, R. 1973 *Practical Histochemistry* John Wiley & sons, London
- Fullmer, H. M. 1966 Histochemical studies of mineralized tissue. *Ann Histochem* 11, 369
- Hallén, O., Brånemark, P.-I., Lindström, J. & Tjellström, A. 1976 Preformed autologous ossicles. Experimental studies. *Acta Otolaryngol* (Stockh) 82, 394
- Heyden, G. & From, S. H. 1969 Histochemical demonstration of ATPase activity during tooth ontogeny in the mouse. *Arch Oral Biol* 14, 225
- Magnusson, B. & Lind, J. 1974 Alkaline phosphatase 5'-nucleotidase and ATPase activity in the molar region of the mouse. *Histochemistry* 42, 221
- Magnusson, B. 1977 Personal communication
- Tjellström, A., Albrektsson, T., Lindström, J., Brånemark, P. I. & Hallén, O. 1977 A clinical pilot study on preformed autologous ossicles. *Acta Otolaryngol* (Stockh)
- A. Tjellström, M.D.  
Dept. of Otolaryngology  
Sahlgrenska Sjukhuset  
S-413 45 Göteborg  
Sweden

## AUDITORY FUNCTION AFTER HAEMOPHILUS INFLUENZAE MENINGITIS

U Rosenhall, O Nylen, J Lindberg and A Kankkunen

*From the Department of Otolaryngology and Audiology Sahlgrenska Hospital  
and the Department of Infectious Diseases University of Göteborg Göteborg Sweden*

(Received April 20 1977)

**Abstract** Eighty three children having recovered from *Haemophilus influenzae* meningitis were examined with audiometrical tests. Fifteen of the children (18.1%) had significant hearing loss. Bilateral severe hearing loss was found in 3 patients. Three patients had severe hearing loss affecting one ear and slight or moderate hearing loss affecting the contralateral ear. Six children had entirely unilateral severe hearing loss. Bilateral or unilateral slight or moderate hearing loss was found in 3 patients. The remaining 68 patients had normal pure tone average. Half of these patients, however, showed minimal hearing impairment at the low and high frequencies.

*Haemophilus influenzae* (HI) is one of the most common etiological agents of bacterial infections in childhood. One of the most serious diseases caused by this species is acute purulent meningitis. The disease is very uncommon in infants under 2-3 months of age, but over that age it is more frequently seen up to 3-4 years of age. It is of importance to establish an early etiological diagnosis so as to minimize both mortality and post infectious sequelae.

There is a high rate of long term sequelae after HI meningitis, even after adequate antibiotic treatment (Sell et al., 1972). Hearing loss seems to be one of the most common sequelae. This article presents the audiological findings of a follow up study of patients with HI meningitis. In a separate article (Lind et al., 1977) the relationship between epilepsy and sequelae will be discussed.

### MATERIAL

During the years 1968-75 altogether 97 individuals were treated for HI meningitis in the Göteborg-Molndal region in Sweden.

The patients were treated either at the Dept of Pediatrics or the Dept of Infectious Diseases, University of Göteborg, or at the Dept of Pediatrics County Hospital, Molndal.

The etiological diagnosis was based on the demonstration of HI in cerebrospinal fluid and/or blood of all but 3 patients. These latter 3 patients, however, had a fourfold or higher antibody titre increase against HI, as measured by a complement fixation test. Three patients died intercurrently. Eighty-three of the remaining 94 patients could be traced and examined with auditory tests. These patients were tested from one month to seven years after the meningitis. The age of the tested individuals at the time of the meningitis varied between 2 months and 22 years, only 3 patients being older than 15 years of age. The mean age of the patients was 3.3 years and the median age 2.4 years.

### AUDIOLOGICAL METHODS

The audiological methods were selected according to the age and development of the

This work has been supported by a grant from the Foundation Tysta Skolan.



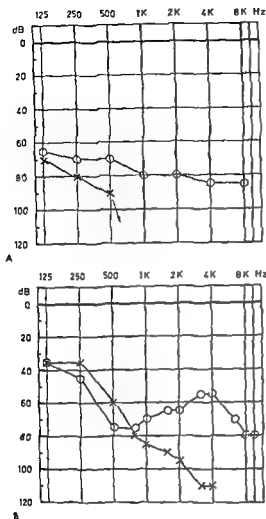


Fig 1 (A, B) Pure tone audiograms of 2 boys: three (A) and six (B) years of age with bilateral severe hearing

loss. Sixteen children, between 6 months and 3-4 years old, were tested with visual reinforcement audiometry (Liden & Kankkunen, 1969). Twenty-six children over 4 years of age were tested with play audiometry according to Barr (1955). Ordinary pure tone audiometry was used in 41 cases.

Patients with a pure tone average (pta) better than 20 dB HL at 500-2000 Hz in both ears were considered to have normal hearing. Thirty-three of the most cooperative patients with normal pta and older than 4 years were picked out in order to detect diminutive hearing impairment. All these patients had normal tympanic membranes. The pure tone thresholds from 125 to 8000 or 10000 Hz were

measured. Speech audiograms were performed in most instances.

A control group consisting of 83 individuals was studied in the same way. None of the individuals in the control group had any history of CNS infections or hearing disorder and there was no sign of middle ear disease at the time of the testing. The age range of the individuals in the control group was 4 to 25 years (mean age 5.6 years, median age 4.7 years of age).

## RESULTS

Fifteen of the 83 patients, 18.1% exhibited different degrees of sensorineural hearing loss (pta  $\geq$  20 dB HL).

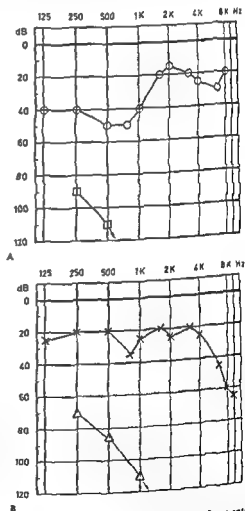


Fig 2 (A, B) Pure tone audiograms of 2 patients: five (A) and five (B) years of age with severe hearing loss in one ear and slight hearing loss in the other.

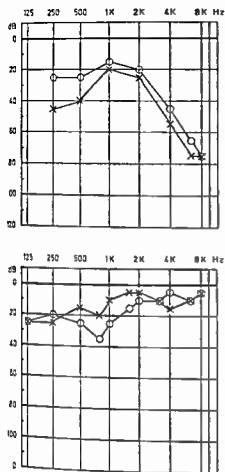


Fig 3 (A, B) Pure tone audiograms of 2 patients two (A) and ten (B) years of age with slight hearing loss

#### Bilateral severe hearing loss

No patient was totally deaf but 3 had severe hearing impairment (pta >60 dB HL) affecting both ears. One of these patients was totally deaf in one ear and his other ear exhibited a hearing loss of 77 dB pta (Fig 1A). The second patient had a hearing loss of 82 dB in one ear and about 70 dB in the other ear (Fig 1B). The third patient gradually developed a severe hearing loss starting about 6 months after the meningitis. His hearing deteriorated to 80 dB in one ear and to 62 dB in the other.

#### Unilateral severe hearing loss

Three patients had unilateral deafness or severe hearing loss combined with a slight or

moderate hearing loss not exceeding 45 dB in the other ear. One of these patients had one deaf ear. His other ear showed a hearing loss of 40–50 dB up to 1000 Hz and of 15–35 dB from 1500 Hz to 8000 Hz (Fig 2A). The second patient had also one deaf ear and a slight hearing loss of 20–35 dB up to 4000 Hz and a high-frequency loss of 60 dB at 8000 Hz in the other ear (Fig 2B). The third patient showed a pure tone average of 65 dB in one ear and of 30 dB in the other ear.

The largest group, consisting of 6 patients, had unilateral severe hearing loss with normal hearing in the other ear. Five of these children had unilateral deafness and one patient had a unilateral hearing loss of about 80 dB.

#### Slight to moderate hearing loss

One patient exhibited a slight sensorineural hearing loss, affecting both ears, with a pta of 20 and 28 dB, respectively. Both ears also showed a high frequency loss of 65–75 dB at 6000–8000 Hz (Fig 3A). Two other patients had a slight unilateral hearing loss. One of them had a pta of 22 dB with a dip of 35 dB at 750 Hz (Fig 3B). Tympanometry and stapedius reflex test were normal. The other had a unilateral hearing loss of 23 dB at 500–2000 Hz and a high frequency loss with a maximum of 60 dB at 8000 Hz.

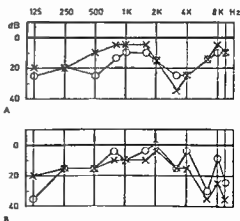


Fig 4 (A, B) Mammal hearing impairment after HI meningitis found in 2 patients ten (A) and nine (B) years of age

### Occurrence of otitic meningitis

An otological examination was performed in the course of the meningitis in 10 of the 15 patients with hearing loss. The tympanic membrane was normal in 7 cases while a slight redness was noticed in 2 cases. One patient was admitted to the hospital with purulent acute otitis media and with signs of purulent meningitis. This patient was the only one in whom the meningitis might be considered secondary to acute purulent otitis media. He became completely deaf in the otitic ear (Fig 2A).

### Minimal hearing impairment

Sixty-eight patients had pta better than 20 dB. Of these individuals, 33 were picked out in order to reveal diminished hearing. Sixteen of these latter patients had pure tone thresholds better than 20 dB at all frequencies tested (125–8000 or 10000 Hz). The remaining 17 individuals exhibited normal hearing capacity at the speech frequencies (500–2000 Hz). They had, however, slight deficiencies reaching or exceeding 20 dB at two or more frequencies outside the speech region.

In many of these cases with minimal hearing deficiencies, the pattern of the pure tone threshold had a serrated appearance. In the low and high frequencies a few, often sharp dips were found, surrounding a plateau of normal hearing at the speech frequencies (Fig 4A, B).

## DISCUSSION

A profound bilateral sensorineural hearing loss is a severe residual after HI meningitis. In our study 3 of the 83 children (3.6%) showed such hearing loss. This figure is comparable to the incidence of bilateral severe hearing loss reported by other authors. Sell et al (1972) found a corresponding figure of 1.8%. Gamsorp & Klockhoff (1974), in their study, reported an incidence of severe hearing loss of

at least 3.5%. In our materials, however, less disabling hearing impairments were more common than bilateral pronounced hearing loss. A considerable discrepancy in hearing between the two ears was found in many patients. A profound unilateral hearing loss combined with only a slight or moderate impairment in the opposite ear was found in 3 cases (3.6%). Still more common was a unilateral profound hearing loss, with normal hearing capacity in the opposite ear, which was found in 6 patients (7.2%). The occurrence of unilateral hearing loss after meningitis has been reported earlier (Grove & Fox, 1949; Trolle 1950; Teng et al, 1962; Sell et al 1972; Landthaler & Andrieu Guirancourt 1974; Johannsen, 1974; Svenungsson et al 1976). Sell et al (1972) found unilateral hearing loss in 8.9% of their cases and Svenungsson et al (1976) in 9% in their study. These results are thus well in accord with the incidence found in the present investigation including 3 cases of slight to moderate hearing loss (one bilateral and two unilateral) the overall incidence of sensorineural hearing loss after HI meningitis was 18.1% in this material. Sell et al (1972) reported a higher incidence of hearing loss, 33.9%. The incidence of severe hearing loss, both bilateral and unilateral was 14.4% in the present study. This figure does not differ markedly from the corresponding incidence reported by Sell et al (1972). Thus in their study, a majority of the patients with hearing loss had only non-significant auditory impairment. Many of their cases were probably similar to those in our study with normal pta in connection with slight hearing impairment in the low and high frequencies. This auditory pattern fits at least 2 cases of non-significant hearing loss in the study of Sell et al. The inclusion of such and similar patients might account for the higher incidence of hearing impairment reported in their study. In the present study as many as half of the patients (51.5%) with normal pta exhibited such minor defects. In the control group similar defects were found in only 10.8%.

Evidently, even those who escape significant hearing loss after HI meningitis often show minor hearing impairment. These deviations, however, are so trifling that they do not constitute a social handicap, and they may not even be noticed by the individuals affected.

Since hearing disorders are commonly found after HI meningitis, all children who have suffered from this disease should be examined with hearing tests. A pure tone audiogram should be performed shortly after recovery. However, hearing impairment may persist long after the meningitis, and so the test should be repeated about 6 months after disease.

## ZUSAMMENFASSUNG

Dreundachtzig Kinder, die nach *Haemophilus influenzae* Meningitis genesen waren, wurden mit audiometrischen Tests untersucht. Fünfzehn der Kinder (18 1%) hatten einen merkbaren Gehörschaden. Doppelseitige schwere Gehörschäden wurden bei drei Patienten gefunden. Außerdem hatten drei Patienten einen schweren Gehörschaden an einem Ohr und einen leichten Gehörschaden am anderen. Sechs Kinder hatten nur einseitige, grave Schwerhörigkeit. Ein leichter Gehörschaden, doppelseitig oder einseitig, wurde in drei Fällen gefunden. Die übrigen 68 Patienten hatten normale Audiogramme. Die Hälfte dieser Patienten hatte aber einen minimalen Gehörschaden, was die niedrigen und hohen Frequenzen anbelangte.

## REFERENCES

- Barr B 1955 Pure tone audiometry for pre-school children. *Acta Otolaryngol* (Stockh) Suppl 121
- Gamstorp I & Klockhoff I 1974 Bilateral severe sensorineural hearing loss after *Haemophilus influenzae* meningitis in childhood. *Neuropadiatrie* 2 121
- Grove W E & Fox M J 1949 Postmeningitic complications with particular reference to otologic sequelae. *Ann Otol Rhinol Laryngol* 58 771
- Johannsen H E 1974 Zur Ätiologie der einseitigen Taubheit im Kindesalter. *HNO* 22 209
- Landthaler G & Andrieu-Guitrancourt J 1974 Contribution à l'étude des ataxies et des surdités secondaires aux méningites purulentes de l'enfant. *Ann Otolaryngol Chir Cervicofac* 91 293
- Liden G & Kankkunen A 1969 Visual reinforcement audiometry. *Acta Otolaryngol* (Stockh) 67 281
- Landberg J, Rosenhall U, Nylen O & Rigner Å 1977 Long term outcome of *Haemophilus influenzae* meningitis related to antibiotic treatment. *Pediatrics* In press
- Sell S H W, Merrill R E, Doyne E O & Zimsky E P Jr 1972 Long term Sequelae of *Haemophilus influenzae* meningitis. *Pediatrics* 49 206
- Svennungsson B, Bengtsson E, Fluor E & Siegborn J 1976 Hearing loss as a sequel to chloramphenicol and ampicillin treatment of *Haemophilus influenzae* meningitis. *Scand J Infect Dis* 8 175
- Teng Y C, Liu J H & Hsu Y H 1962 Meningitis and deafness. Report of 337 cases of deafness due to cerebrospinal meningitis. *Chinese Medical Journal* 81 127
- Trolle E 1950 Defective hearing after meningococcal meningitis. *Acta Otolaryngol* (Stockh) 38 384
- U Rosenhall M D  
Audiologiska avd  
Öronklinik  
Sahlgrenska sjukhuset  
S-41343 Göteborg  
Sverige

## EPITHELIAL MIGRATION ON THE TYMPANIC MEMBRANE

*An Experimental Study*

D Boedts and W Kuypers

*From the ENT Department U I A University of Antwerpen Wilrijk (Antwerpen) Belgium and  
the ENT Department University of Nijmegen Nijmegen The Netherlands*

(Received May 10 1977)

**Abstract** The existence of an epidermal migration pattern was studied in mice using a whole mount autoradiographical technique. The mitotic activity appeared to be confined to an area close to the annulus tympanicus. A very slow migratory activity of the labelled cells was observed situated in the basal cell layer. Perforation of the tympanic membrane showed no effect on the position of the labelled cells. This slow migration was considered to differ from the rapid epithelial migration described by several authors. The latter migration was suggested to represent a mechanism for cleaning the tympanic membrane.

The existence of an epidermal migration pattern on the human eardrum was suggested as early as 1877 by Burnett and confirmed by Buck (1880) and Bezold (1908). They supposed that this cell migration might explain the self-cleaning property of the external meatus. This phenomenon was described in greater detail on the human tympanic membrane with the use of ink dots by Stinson (1936).

From comparable experiments, Magnoni (1938) concluded that cholesteatoma of the middle ear could not originate from the inward migration of epithelium from the external meatus, since no migration of epidermal cells was noted in this direction. In contradiction Simmons (1964) advocated the use of the ink dot marking technique as a clinical test for the detection of cholesteatoma formation in central perforations. A careful analysis of ink dot migration patterns and rates in human tympan-

ic membranes was made by Albert (1964). He noted an average migration rate of 0.07 mm per day, while the umbo appeared to be the centre of migration. The latter author also doubted on the existence of any relationship between epidermal migration and the origin of middle ear cholesteatoma because such migration would tend to prevent rather than cause such an epidermal cyst. Tomasetti (1965), Franz (1966) and Gulzow (1973) arrived at same conclusions with comparable experiments.

Using a radioactive tracer and DNA precursor ( $H^3$  thymidine) Litton (1968) identified and traced the route of the labelled nuclei of the squamous epithelium of the eardrum and external auditory canal of guinea pigs. He concluded from his experiments that in guinea pigs the epidermal migration occurs by a differential growth rate in an area of the auditory canal wall just distal to the annulus tympanicus. This very rapidly proliferating generation centre should maintain a thin pliable lining over the vibrating tympanic membrane.

Similar experiments on traumatic eardrum perforations (Clawson & Litton 1971) give support to this hypothesis of a differential growth rate from a generation centre.

Apart from these observations several questions concerning this migration phenomenon remain and require further research. The aim

this paper therefore is to discuss results of series of experiments on this migration phenomenon with the use of autoradiography

## METHODS

### *Ink dot marking*

Small Indian ink dots were placed at various locations on the tympanic membrane and the rim of the external auditory canal of adult animals (20 animals)

For this marking procedure, using the operating microscope, the animals were anaesthetised with nembutal (60 mg/kg, administered intraperitoneally)

### *Autoradiography*

In the isotope experiments were performed on two series, each consisting of 8 Swiss albino mice. With the use of the operating microscope a small perforation was made in the right eardrum of the anaesthetised animals. The left ear served as control. Immediately after perforation, the animals received 1  $\mu$ Ci  $^3$ H-thymidine/g body weight administered intraperitoneally.

In order to exclude any possible influence of the diurnal rhythm on the final results one group of animals was injected at 12.00 hours and another group at 24.00 hours. The animals were sacrificed at different intervals varying from 2 hours up to 18 days. The tympanic membranes were dissected, fixed in neutral formaldehyde and placed on gelatinized slides, with the epidermal layer upside.

After dehydration and cleaning in xylol alcohol solutions the slides were coated with a nuclear emulsion (NTB<sub>2</sub>, Eastman Kodak) using the dipping technique (Kopriwa & Leonard 1962). The specimens were stored in lightproof boxes for 2-4 weeks and subsequently developed, fixed and stained with hematoxylin-eosin.

The exact localization of the labelled nuclei was registered using a lateral drawing tubus, viewed on the microscope. Only those cells where the deposit of silver granules was defi-

nately separable from the background were scored.

Apart from the use of  $^3$ H-thymidine, similar experiments were performed with the use of 5-iodo-2-(deoxy)-6 $^3$ H-uridine (IDU). Thymidine is in fact not an ideal DNA precursor in long term turnover experiments, because of the reutilization and reincorporation of labelled breakdown products. This is not the case for IDU, where the methyl group in position 5 is replaced by iodine. Once incorporated, IDU remains fixed in the DNA molecule through the whole lifetime of the cell, and reutilization after cell-death is minimal and ineffective. According to Commerford (1965) and Feinendegen et al. (1966) IDU is an excellent substance for turnover experiments in the long term.

One disadvantage is the fact that IDU is less easily incorporated than thymidine (Hughes et al., 1964).

In two series, each consisting of 6 mice, the animals received 5  $\mu$ Ci IDU per gram body weight, injected intraperitoneally. The animals were sacrificed at different intervals and their tympanic membranes were studied according to the technique used for  $^3$ H-thymidine.

## RESULTS

### *Ink dot marking*

The results of this preliminary experiment were disappointing. Because of the rapid disappearance of the dots, no migratory pattern could be observed. In 76 ears studied with this method the ink dots had already disappeared after one or two days.

### *Autoradiographs*

The results obtained from normal tympanic membranes are summarized in the drawings of the tympanic membranes (Figs 1 and 2). Each dot in these figures represents one labelled cell. The highest mitotic activity in the pars tensa is mainly situated near the annulus tympanicus and the manubrium mallei.

In the pars flaccida no specific localization

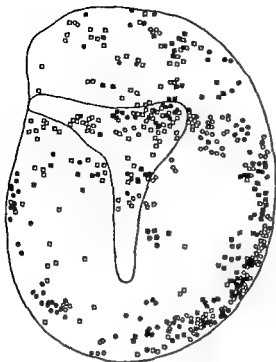


Fig 1 After 2 hours (●) after 1 day (○) after 2 days (□) after 4 days (■)

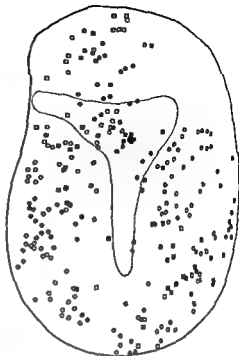


Fig 2 After 8 days (○) after 12 days (●) after 14 days (□) after 18 days (■)

of the labelled cells was found. During an observation period of 18 days no clear migration pattern could be found. Only a slight tendency of the labelled cells to move slowly from the periphery to the centre of the pars tensa was observed. With progression of time the labelling became more difficult to identify because of the dilution of the isotope by cell division or physiological destruction.

The data obtained with the perforated tympanic membranes (Fig 3) clearly show that no essential difference can be observed in the isotope distribution in comparison with the control ears. The perforations seem to close without any tendency of the labelled cells to migrate towards the perforation.

The results obtained with  $H^3$ -IDU appeared to be fully comparable to those after  $H^3$ -thymidine administration (Fig 4).

## DISCUSSION

According to various authors there undoubtedly exists a migratory activity of epidermal cells

on the human tympanic membrane (Stinson 1936, Magnoni 1938, Simmons 1961, Lintz 1963, Alberti 1964, Tomasetti 1965, Franz 1966, Gölzow 1973).

Several mechanisms underlying this migration have been postulated, such as the vibration of the tympanic membrane or the blood flow in the vessels of the membrane (Lintz 1952).

According to Litton (1968) and Clawson & Litton (1971) a generation centre of epithelial cells, localized in the external meatus and close to the annulus, should be the driving force for this migration phenomenon in guinea pigs. It takes about 13 days to cross the tympanic membrane completely. According to the same authors a comparable mechanism should exist in human.

In contrast to the findings of these authors our results failed to show the existence of such a rapid epithelial cell migration in mice. Only a very slow movement of labelled cells was observed from the annulus towards the central parts of the pars tensa. Even after 18 days

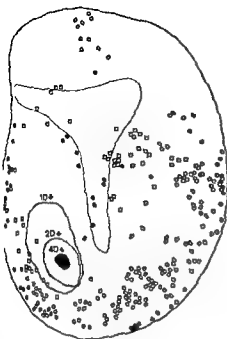


Fig 3 After 1 day (●) after 2 days (○) after 4 days (■)

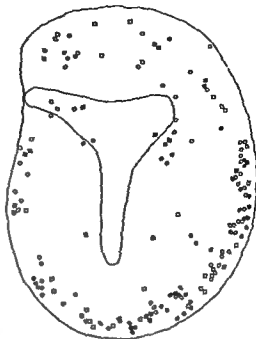


Fig 4 After 2 hours (○) after 2 days (●) after 4 days (■) after 8 days (◆)

tany labelled cells are still distributed arbitrarily on the whole tympanic membrane

These experiments reveal the existence of a centre of high mitotic activity in the annular area and confirm the findings made by Reyten & Kuypers (1971). The slow migration appeared to be mainly confined to the cells of the basal epithelial layer. It seems unlikely, however, that this migration is comparable to the migratory activity described by Litton (1968) and Clawson & Litton (1971).

It is highly likely that the observations made by Litton in guinea pigs (1968) represent the migration of a very superficial layer of the epithelium. One argument in favour of this hypothesis is the similarity of the results they obtained in using Indian ink labelling and  $H^3$  thymidine. With ink labelling only the upper part of the stratum corneum can be marked. Furthermore the thickness of the tympanic membrane of the guinea pig varies between 20 and 40  $\mu\text{m}$  and can increase to more than 100  $\mu\text{m}$  after perforating. Because the penetration of  $H^3$  radiation is maximally about 8  $\mu\text{m}$ , la-

belled cells in the basal cell layer of the guinea pig tympanic membrane cannot activate the emulsion layer in whole mount autoradiography. Therefore the observed blackening in the emulsion must originate from the superficial layers of the epithelium. In mice, however, the thickness of the membrane varies between 5 and 10  $\mu\text{m}$ . So the radiation from labelled basal cells in our experiments will activate the emulsion in whole mount autoradiography. The results obtained clearly demonstrate that perforation does not influence the position of the labelled cells to any great extent.

The migration of the upper layer of the stratum corneum might represent a physiological mechanism taking care of the removal of keratin and cerumen from the tympanic membrane. The displacement and even extrusion of eargrommets might be at least partly attributable to this mechanism. The driving force of this cleaning process remains a matter of speculation, however.



(•6757)

DPR + OKN

DPR + OKNRD

Fig 1 This figure shows nystagmus recordings of one squirrel monkey in two different conditions: rotation in light (DPR + OKN) and rotation with a mirror in light (DPR + OKNRD). In this monkey the turning points of nystagmus direction (arrows) are clearly delayed (phase lag) in DPR + OKNRD, but nystagmus rhythm is almost regular.

tubular stimuli in order to investigate possible mechanisms associated with this phenomenon.

### METHODS

Healthy adult male or female squirrel monkeys (body weight 450–700 g) were used. Rotatory stimuli were provided with a pendular rotation table suspended by a stainless steel wire from a 4 meter high ceiling. When the table was rotated  $180^\circ$  and released, one oscillation took 15.5 sec. Eighteen cycles were obtained in this manner with a gradual damping motion observed from the initial release. The maximum angular acceleration of the initial oscillation produced by the present apparatus was  $29^\circ/\text{sec}^2$ , and the maximum angular speed was  $72^\circ/\text{sec}$ .

To provide pendular rotation and optokinetic stimuli simultaneously, a 1 meter diameter white cylinder with 16 black stripes (3.5 cm wide) was installed around the pendular rotation table. To eliminate peripheral vision during optokinetic stimulation, a head cover with an opening in front of the subject's eyes was used.

Visual-vestibular conflict was accomplished by reversing the direction of the optokinetic stimulus with a mirror installed at a  $45^\circ$  angle in front of the monkey's eyes. Because the distance between the eyes and the cylinder surface became longer and also the angle be-

tween the subject's visual axis and the cylinder surface became obtuse, the optokinetic stimulus magnitude was reduced to approximately 70%.

Horizontal and vertical eye movements were recorded on a Beckman dynograph through a d.c. amplifier. The position of the pendular rotation table was recorded by a potentiometer which was installed at the bottom of the table.

Each monkey was secured in a laboratory built restrainer, 0.5 mg/kg amphetamine was injected intramuscularly, and the test was begun about 15 min later.

Testing was comprised of three conditions. First, vision was excluded and damped pendular rotation (DPR) was applied. Second, the monkeys were exposed to both rotation and the optokinetic stimulus: rotation in light (DPR + OKN). Third, each subject experienced pendular rotation with the visual field reversed, rotation in light with a mirror (DPR + OKNRD). The tests were performed following the above described order of 1 through 3, one test a day each, except for the training phase in which DPR + OKNRD was repeated twice a day.

The first part of the study covers the analysis of DPR + OKNRD and comparison between DPR + OKNRD and DPR. Furthermore, the training effect and effect from vestibular deafferentation (labyrinthectomy or lateral

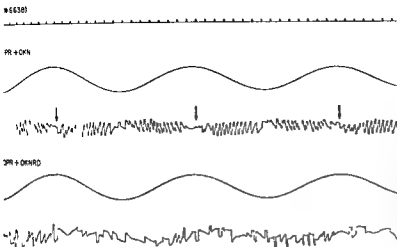


Fig 2 This subject shows very irregular saccadic or slow eye movement in rotation with a mirror in light (DPR+OKNRD). It is impossible to decide direction changing point of nystagmus.

semicircular canal plugging) in DPR+OKNRD situation were studied.

Upon the completion of the functional experiments, all of those operated animals were perfused intracardially and serial sections of the temporal bones were prepared according to standard procedure.

## RESULTS

### DPR+OKNRD

The purpose of this part of the experiment was to evaluate the squirrel monkey's response to vestibular-visual directional conflict situation. In order to eliminate any training or habituating effect, the data analysed for this portion of the study were taken from the initial trials of 17 squirrel monkeys only.

In those monkeys who could resolve the vestibular-visual conflict, the direction of nystagmus response was determined by the optokinetic stimulus. The table's turning point and nystagmic direction changing point were fairly well matched, and nystagmic rhythm was regular.

On the other hand, those monkeys who could not pursue the visual target properly showed a phase lag between the table's turning point and nystagmic direction changing point (Fig 1). In addition, many of these subjects

exhibited irregularity in nystagmic rhythms (Fig 2). Many also floundered, indicating that the condition was stressful to them.

Among the total of 17 subjects in this experimental group, 2 demonstrated very little phase lag, or irregularity of nystagmic rhythm. Eight exhibited either relatively large phase lag but with good nystagmic rhythm or minimal phase lag with considerable irregularity in the nystagmic rhythm. The remaining 7 showed either very large phase lag, or very severe irregularity in nystagmic rhythm. Therefore, 15 out of 17 (88%) showed poor target pursuing ability, when the directions of optokinetic stimulus and vestibular stimulus were conflicting.

The phase lag and the irregularity of nystagmic rhythm did not necessarily show an identical tendency. The range in phase lag among different monkeys is shown in Fig 3. As a trend, when the stimulus magnitude was reduced through the damped oscillation of the table, the phase lag became less. However, the degree of phase lag reduction was not necessarily parallel to that of stimulus magnitude reduction.

Those monkeys with good target pursuing ability (in DPR+OKNRD) showed minimal phase lag even at the first oscillation. In contrast, subjects with poor target pursuing ability showed considerable phase lag even when

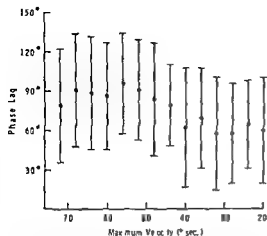


Fig 3 The means and standard deviations (17 squirrel monkeys) of phase lag in DPR+OKNRD. Abscissa: phase difference between direction changing points of table and nystagmus. Ordinate: maximum stimulus velocity of each oscillation.

stimulus magnitude became small. In many of those, bidirectional irregular eye movements or nystagmic wave forms were observed.

According to this result, the monkeys who could pursue the visual target in DPR+OKNRD condition were rather exceptional when compared with the majority which could not overcome the vestibular-visual directional conflict, even though the vestibular input was minimal. On the other hand, during ordinary pendular rotation in light (DPR+OKN), all monkeys showed no difficulty in pursuing the visual target with no phase lag or irregular nystagmic rhythm. All monkeys in this series showed good vestibular nystagmus in DPR (without vision), therefore all of them had good vestibular end organ functions.

#### Comparison Between DPR+OKNRD and DPR

It is now known that the crista ampullaris lateralis acts as a change of speed receptor in response to sinusoidal rotation within a certain periodicity (Melvill Jones & Milsum, 1971). The vestibular-visual conflict situation in this experiment consisted of not only opposite directions of visual and vestibular

stimuli, but also included the ratio between the two stimulus magnitudes. Among the 17 squirrel monkeys, 8 were randomly selected for studying the relation of strength of vestibular nystagmus and pursuing ability of OKNRD. The average eye speed from these 8 monkeys when rotated in light (DPR+OKN) or in dark changed linearly (Fig 4) and the variances in both conditions were somewhat similar. (Open circles in Fig 4 represent the data taken from a subject with complete lateral semicircular canal block on one side and incomplete canal block on the other side.) The average eye speed of DPR in each oscillation was between 53.3 and 73.8% (average 65.1%) of that of DPR+OKN. This value reduced according to the stimulus decline. When a mirror was installed (DPR+OKNRD), the optokinetic stimulus speed reduced to about 70% of the stimulus speed without a mirror. Thus, the eye speed of DPR should be about 93% ( $65.1\% \times 100/70$ ) of that of DPR+OKNRD, if the monkeys were able to pursue the OKNRD well.

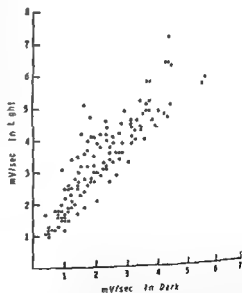


Fig 4 This scatter graph shows slow phase eye speed for comparing the strength of vestibular nystagmus (DPR, abscissa) and optokinetic nystagmus (DPR+OKN, ordinate). Closed circles represent 8 normal monkeys. Open circles are recorded from the monkey in which one lateral semicircular canal is completely blocked whereas the contralateral one is incompletely blocked.

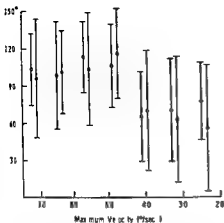


Fig 5 The means and standard deviations of phase lag obtained from 6 monkeys for the study of training effects. Open and closed circles represent the first and last trials respectively.

When the nystagmic eye speed in DPR, and that in DPR+OKN situation were compared in each individual monkey, the former was 49.3–89.5% of the latter. The inter-individual variance was large. Therefore, when the vestibular provoked nystagmic response and the target pursuing ability in DPR+OKNRD situation were compared, no correlation existed between those two.

### Training Effect

For this part of the experiment 8 squirrel monkeys with poor visual target pursuing ability in DPR+OKNRD situation were selected and the test trials were performed twice a week (2 tests per day) for 5 weeks (total 20 tests) in order to investigate if any functional improvement existed. As was stated in the previous section, amphetamine was administered to maintain monkeys alert during this sort of unnatural stimulus condition. To avoid the amphetamine's cumulative effect, training trials had to be spaced no more than twice a week.

One subject in this group showed slight improvement (17%); one subject's response became worse while the others exhibited basically no difference between the first test and the last test. Inter-individual variance in

creased (Fig 5). The monkey which showed some improvement also showed variable results and did not depict any gradual shortening of the phase lag.

### Vestibular Deafferentation

#### A Labyrinthectomy

Three squirrel monkeys (randomly assigned) received bilateral labyrinthectomies (two staged). Four months after unilateral labyrinthectomy (first stage), a very weak spontaneous nystagmus was still observed when the subject was placed in the dark (with amphetamine injection) and slight directional difference (and a reduction of phase lag) was found in the DPR+OKNRD test situation (Fig 6). When the direction of spontaneous nystagmus and OKNRD matched, the pursuing ability was slightly improved.

Immediately after the second stage labyrinthectomy (about four months after the unilateral labyrinthectomy), the phase lag previously exhibited in the DPR+OKNRD test situation was clearly reduced or even disappeared (Fig 6). However, a reduction of nystagmic eye speed was also noted in this situation.

The eye speed of the vestibular nystagmus

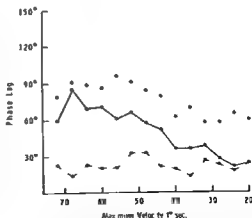


Fig 6 This figure shows the decrease of phase lag (mean better optokinetic pursuit) in DPR+OKNRD in labyrinthectomized subjects. Closed circles are the mean of 17 intact monkeys. Open circles connected by solid and interrupted lines represent the post-unilateral and bilateral labyrinthectomy status respectively.

BLATERAL LATERAL SEMICIRCULAR CANAL BLOCK (TWO STAGE)  
IN DS PQ. (#5764)

DPR+OKN



DPR+OKNRD



Fig 7 Nystagmus recordings of the monkey in which both lateral semicircular canals are completely blocked. Optokinetic pursuit in DPR+OKNRD is perfect and phase lag appeared.

(DPR) after the unilateral labyrinthectomy reduced to about 60%, and after bilateral operations the DPR nystagmus was abolished. In the test situation of DPR+OKN, the eye speed was not changed after the unilateral labyrinthectomy, but was lower after the bilateral labyrinthectomies, as the pursuing ability declined due to the disappearance of the vestibular inputs.

### B Lateral semicircular canal blocking

Another 3 squirrel monkey subjects (randomly selected) were used for this part of the experiment. The tests were performed 1 week and 2 weeks after unilateral lateral semicircular canal plugging. Four weeks after the first operation the contralateral canal blocking was performed. Thereafter, the test was similarly performed 1 week and 2 weeks after the second operation.

The eye speed of vestibular nystagmus evoked by DPR after unilateral operation was about 60% of that observed pre operation (somewhat similar to the situation after the unilateral labyrinthectomy). Following the blocking of the contralateral lateral semicircular canal the DPR evoked nystagmus completely disappeared, except for 1 monkey which had incomplete obliteration of the semicircular canal on the second operation side. Histological examination indicated that in 2

animals bilateral horizontal semicircular canal plugging was complete, while in the third animal, the contralateral (second operation) canal block was incomplete. Nystagmus evoked in the test situation of DPR+OKN showed no obvious difference between pre operation, post unilateral operation and post bilateral operation (3 monkeys).

The 2 squirrel monkeys with the

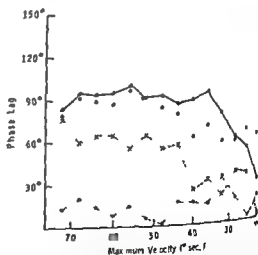


Fig 8 This figure shows the phase lag decrease in bilateral horizontal semicircular canal blocking in OKNRD test. Open circles connected with solid lines represent the post unilateral and bilateral operation. One monkey with incomplete block is not included.

lateral lateral semicircular canal plugging, showed significant improvement of target tracking ability and a disappearance of phase lag (in the test situation of DPR+OKNRD) (Fig 7). This change was not observed following the unilateral operation (O—O in Fig 1). In the incomplete obliteration monkey, the eye speed of vestibular nystagmus (DPR) was about 38% of that of the optokinetic nystagmus (DPR+OKN) (O in Fig 4). This scalar value was lower than the lowest value (49.3%) observed in the 8 normal control subjects. However, the target pursuing ability was still incomplete in the test situation of DPR+OKNRD, and the result (X---X in Fig 8) was found just in between unilateral canal blocking and bilateral canal blocking (O—O).

Unlike the post bilateral labyrinthectomy condition the results from the bilateral lateral semicircular canal block indicated that the postoperative eye speed in the DPR+OKN test situation did not decline. The target pursuing ability in the DPR+OKNRD test was found to be good after successful bilateral canal block (Fig 7).

## DISCUSSION

Results from the present experiment indicate that the majority of normal squirrel monkeys employed could not control unfavorable vestibular input when its direction was in conflict with that of visual information (DPR+OKNRD).

As far as the visual stimulus is concerned, the maximum angular acceleration produced by this DPR apparatus was  $29^\circ/\text{sec}^2$  and the maximum angular speed was  $72^\circ/\text{sec}$ , which should not be difficult for squirrel monkeys to pursue visually. As was found in the animals with bilateral semicircular canal blocking, no difficulty was encountered in pursuing the visual field without having dynamic semicircular canal inputs in the test situation of DPR+OKN.

Fukuda and others (1957), using rabbits,

reported vestibular-visual coordination by exhibiting the improvement of the visual target tracking ability with the supplementation of  $1^\circ/\text{sec}^2$  angular acceleration to the vestibular apparatus. Later, Veenhof (1965) found similar results by supplementing linear acceleration. However, it should be considered that the rabbit, having laterally positioned eyes, has a different oculomotor system compared with that of the primate.

When optokinetic stimulus is presented with an angular acceleration of  $1^\circ/\text{sec}^2$ , the adaptation limit is somewhere around  $120^\circ/\text{sec}$  in the squirrel monkey. Except for the fact that the stimulus was sinusoidal in nature, the maximum stimulus speed of  $72^\circ/\text{sec}$  in the present DPR stimulus is definitely below this limit, and therefore, as was observed monkeys should not have any difficulty in tracking the target. On the other hand, only two subjects could pursue the visual target in the test situation of DPR+OKNRD, and inter-individual variance was remarkably large among all monkeys tested. Even in poorly pursuing monkeys, vestibular nystagmus was modified in this test condition suggesting that visual input is dominant.

Gonsker & Melvill Jones (1976) reported that when prisms were installed in front of the eyes, in order to reverse left-right directions in humans and cats, the subjects showed poor target pursuit ability at the early stage when sinusoidal rotation was employed. At least in the initial stage of direction reversal, vestibular-visual conflict clearly existed in both humans and cats.

Guedry (1968) studied the relationship between the visual acuity and vestibular stimulation or vestibular nystagmus, and reported the existence of dominant inter individual variance of visual acuity decline. He also noticed the correlation between the degree of vestibular nystagmus in light and visual acuity during the rotation. The degree of visual input dominance over the vestibular evoked nystagmus showed a large inter individual variance. From our observation in squirrel monkeys,

no clear relationship existed between the target pursuing ability in DPR+OKNRD test and the degree of vestibular evoked nystagmus (DPR). It is considered that target pursuing ability is dependent on the degree of visual input dominance over the vestibular input.

Young et al (1973) reported that the circular vection was enhanced when the directions of vestibular and visual stimuli matched, and contrarily circular vection was reduced when the directions did not match. Under a direction matching situation, the responses were summated, however, when the stimuli conflicted, the qualitative nature of the response changed. Although circular vection and nystagmus are not exactly similar responses, our experiment demonstrated one aspect of vestibular-visual conflict.

Under the present experimental situation of DPR+OKNRD, the qualitative change of the nystagmus was quite common in addition to the phase lag. Irregular change of nystagmic direction and irregular eye movement (wave form) were commonly observed. The former may represent non linearity in the management of conflicting stimuli, while the latter may represent the central confusion failing to manage direction contradicting inputs.

It is generally accepted that a visual stimulus is dominant over a vestibular stimulus. It is known that ballet dancers and figure skaters can inhibit post-rotatory nystagmus by visual fixation, but this ability is acquired after training. Untrained people usually cannot suppress vestibular reaction well. In their chicken rotation experiments, Fukuda and others (1958), reported that in order to control vestibular inputs, transition occurred in posture from a passively rotated condition to an actively adjusting status after repeated rotatory training. In such active postural adjustment, muscle and joint inputs should also make an active contribution.

The test situation of DPR+OKNRD, visual stimulus and vestibular stimulus were given in conflicting directions, was an unnatural stimulus situation for the body. However, even un-

der this sort of conflicting situation, Gershner & Melvill Jones (1976) exhibited adaptation could still occur. The inhibition of vestibular nystagmus and change in phase shift were observed. This was based upon somewhat altered stimulus management mode which could be considered as a system plasticity. The repeated application of optokinetic stimulus resulted in response enhancement; however, in the situation of vestibular-visual conflict, which was an unfavorable and unexperienced condition for the body, adaptation may not occur easily.

As was seen in our one case with unilateral complete semicircular canal block and contralateral incomplete canal block, even though the vestibular input was much reduced, the vestibular-visual conflict could not be controlled easily in the test situation of DPR+OKNRD. This result indicates that the conflict could be established even by limited vestibular input.

The different control ability of vestibular-visual conflict among different squirrel monkeys was observed in the present experiment. Two monkeys with good pursuing ability in the present series showed good OKN tracking ability, good DPR nystagmus, and good post-rotatory nystagmus. One interesting finding in those was that the duration of post-rotatory nystagmus and optokinetic after-nystagmus was relatively long and the declining pattern was very gradual. It is not known whether the ability to control vestibular-visual conflict and system adaptability are similar in nature or not, but the control of vestibular-visual conflict might be something different from the ability obtained through repeated training, even though the mode of training could be another crucial factor.

## ZUSAMMENFASSUNG

Im Totenkopffaffen wurde der Richtungskonflikt zwischen den vestibulären und visuellen Reizen untersucht. Der Affe wurde sinusförmig gedreht und die Richtung des Zielverfolgung wurde vor seinen Augen umgekehrt. Der Einbau eines Spiegels auf einer 45° Ebene. Eine ge-

duelle Abweichung wurde beim Kontrollieren vestibular-visuellen Konflikts bemerkt. Der Grad des vestibular-visuellen Konflikts und der Reizgröße hatte lineare Beziehung. Gleichfalls bestand keine Beziehung zwischen dem vestibular-visuellen Konflikt und Anreizung des vestibulären Nystagmus. Während Zustandes des vestibular-visuellen Konflikts veresserte sich die visuelle Zielverfolgung nicht, wenn mal am Tage Versuche unternommen wurden, und war zweimal in der Woche, auf die Dauer von fünf Wochen. Die Verminderung der vestibulären Zufuhr, die durch Operation geschaffen wurde, führte zu einer Verminderung des vestibular hervorgerufenen Nystagmus und des vestibular-visuellen Konflikts. Deren Grade waren jedoch nicht unbedingt parallel. Ein interindividueller Unterschied in der Reaktion auf den vestibular-visuellen Konflikt wurde auf die vestibular-visuelle Koordination bezogen.

## REFERENCES

- Davis K & Melvill Jones, M 1976 An adaptive neural model compatible with plastic changes induced in the human vestibulo-ocular reflex by prolonged optical reversal of vision *Brain Res* 103 546
- Dichgans J & Brandt Th 1972 Visual-vestibular interaction and motion perception *Bibl Ophthalmol* 82, 327
- Dichgans J, Schmidt C L & Graf, W 1973 Visual input improves the speedometer function of the vestibular nuclei in the goldfish *Exp Brain Res* 18 319
- Fukuda T, Hinoki, M & Tokita T 1957 Provocation of labyrinthine reflex by visual stimuli *Acta Otolaryngol* (Stockh) 48 425
- 1958 Static and kinetic labyrinthine reflex *Acta Otolaryngol* (Stockh) 49, 467
- Gonshor, A & Melvill Jones, G 1976 Short term adaptive changes in the human vestibular-ocular reflex *arc J Physiol* 256 361
- Grusser, O J & Grusser Cornhels, U 1972 Interaction of vestibular and visual inputs in the visual system. In *Progress in Brain Research Vol 37 Basic Aspects of Central Vestibular Mechanisms* (ed A Brodal & O Pompeiano), Elsevier Amsterdam pp 573-583
- Guedry, F E Jr 1968 Relations between vestibular nystagmus and visual performance *Aerospace Med* 39 570
- Henn, V, Young, L K & Finley, C 1974 Vestibular nucleus units in alert monkeys are also influenced by moving visual fields *Brain Res* 71 144
- Klinke, W & Schmidt C L 1970 Efferent influence on the vestibular organ during active movements of the body *Pflugers Arch* 318 325
- Melvill Jones, G & Davis P 1976 Adaptation of cat vestibulo-ocular reflex to 200 days of optically reversed vision *Brain Res* 103 551
- Melvill Jones, G & Milsum, J H 1971 Frequency-response analysis of central vestibular unit activity resulting from rotational stimulation of the semicircular canals *J Physiol* 219 191
- Veenhof V B 1965 On the influence of linear acceleration on optokinetic nystagmus *Acta Otolaryngol* (Stockh) 60 339
- Young, L R, Dichgans J, Murphy, R & Brandt Th 1973 Interaction of optokinetic and vestibular stimuli in motion perception *Acta Otolaryngol* (Stockh) 76 24

M Igarashi M D  
Dept of Otorhinolaryngology  
and Communicative Sciences  
Baylor College of Medicine  
Houston TX 77030 USA



## NEURAL DISCHARGE OF MEDIAL GENICULATE BODY UNITS AND SINGLE SEMICIRCULAR CANAL STIMULATION

D. Troiani, L. Petrosini and E. A. Palestini<sup>1</sup>

*From the Institute of Physiology Università Cattolica S. Cuore Rome Italy*

(Received March 7 1977)

**Abstract** In curarized guinea pigs 88 neurons of the medial geniculate body (MGB) were tested with vestibular and acoustic stimulations. Single semicircular canals were stimulated thermally. Convergence of acoustic and vestibular afferences on the same MGB unit was observed. Following stimulation of the semicircular canals activation and inhibition of unitary discharge were recorded, inhibition being predominant while when clicks were delivered bursts of activity occurred. The implications of MGB in vestibular and acoustic integration are postulated.

Attempts have been made to define, for several species, specific thalamic cell groups carrying vestibular information to vestibular cortical fields identified within the somatosensory field SI and within a parietal field in carnivores and primates (Fredrickson et al., 1974; Liedgren & Schwarz, 1976, for recent reviews). Cells responding to vestibular stimuli appeared to be scattered over large thalamic areas, known for other functions (Copack et al., 1972; Deecke et al., 1973, 1974; Germandt, 1950; Hassler, 1948, 1964, 1972; Mickle & Ades, 1954; Raymond et al., 1974; Sans et al., 1970; Spiegel et al., 1965; Wepsic, 1966), the ventro-basal and posterior thalamic nuclei, however, seem to receive relatively direct vestibular projections (Deecke et al., 1974; Liedgren & Schwarz, 1976; Liedgren et al., 1976).

In the guinea pig, the vestibular cortical field is located within the somatosensory fore

limb area SI (Ödkvist et al., 1973) but the thalamic stations on the vestibulo-cortical pathway have not been clearly identified although vestibular projections have been described within the *nucleus parafascicularis*, lateral and medial geniculate bodies (Geretsoff, 1950). The medial geniculate body (MGB) receives the greatest number of vestibular fibres and, consequently, it has been proposed as the major vestibular projection area.

The purpose of this study was to verify the vestibular representation in MGB and to analyze the possible convergence of inputs from single semicircular canals on these diencephalic neurons. Acoustic and vestibular convergence was also investigated. Previous studies on other structures connected with the vestibular system (lateral vestibular nucleus, paramedian pontine reticular formation, oculomotor nucleus, nystagmogenic cortex area) have been carried out in the guinea pig and various patterns of convergence of ampullar afferences have been demonstrated following thermal stimulation of single osseous canals (Desole & Palestini, 1969; Manna & Desole, 1966; Palestini & Desole, 1971; Petrosini et al., 1975).

This research was supported by a grant of CNR.  
<sup>1</sup> Ear, Nose and Throat Department, Ospedale S. Matteo, Genoa, Italy.



Fig. 1. Electrolytic lesion of a recorded site in the left medial geniculate body (MGB) (arrow). rn, red nucleus; III, oculomotor nucleus. Magnification  $\times 10$ .

## METHODS

Twenty guinea pigs of both sexes, weighing 350–500 g were used in the present research. The animals were initially anaesthetized with ether, then tracheotomized and fixed in the prone position in a stereotaxic apparatus with the head tilted slightly down. The animal was maintained throughout the experiment at  $37-37.5^{\circ}\text{C}$  temperature by heat lamp and pad. The ECG was monitored by means of needle electrodes inserted into the left and the right forelimbs. The heart rate was roughly 160 beats/min initially, with a tendency to fall slightly as the experiment progressed. Two holes were drilled bilaterally in the temporal bones: the first rostro dorsally and the second just posterior to the external auditory meatus, for epitympanic recessus and *bulla tympanica* opening. Thus, it was possible to expose the iliac of the lateral and anterior semicircular osseous canals in the epitympanic recessus and the prominence of the posterior semicircular osseous canal on the medial wall

of the *bulla tympanica*. Thermal stimulus was applied, employing the technique of Lorente de No (1931), on the single osseous ampulla of both lateral and anterior canals and on the prominence of the posterior canals, by means of a silver electric thermode (Mancinelli, 1975). The caloric stimulus was controlled in order to induce in the heated ampulla an increase in temperature of maximum  $2^{\circ}\text{C}$ . This temperature difference always induced eye nystagmic beating on the plane of the stimulated canal (Flourens 1842).

In the absence of spontaneous eye nystagmus, the animals were curarized with 1–2 mg of Tubocurarine HCl and artificially ventilated. Under surgical anaesthesia, following appropriate craniotomy, the temporoparietal lobes and the occipital poles were removed by suction, in order to expose the dorsal surface of posterior thalamus, the IIIrd ventricle and the *corpora quadrigemina* which were carefully protected with warm mineral oil maintained at a constant temperature of  $37.5^{\circ}\text{C}$ . The wound edges and the pressure points of

the stereotaxic apparatus were repeatedly infiltrated with Xylocaine. In order to record unitary discharges, a tungsten microelectrode (tip diameter 1–4  $\mu\text{m}$ , resistance 600–1000 K $\Omega$ ) was driven by means of a micromanipulator into the medial geniculate bodies and in few experiments, in the *brachia colliculi inferioris*, in the inferior colliculi and in the VIIIth vestibular nerve. The recording microelectrode was connected through a P5 Grass preamplifier to a Tektronix 565 dual-beam oscilloscope and to a Hewlett-Packard 3960 instrumentation recorder.

In order to evaluate the possible spread of thermal stimulus applied to a single semicircular canal, a thermocouple was placed in the neighbouring ampulla. Negligible changes in temperature (0.1–0.5°C) were recorded. In some experiments, tests were performed to ascertain whether this temperature change was capable of modifying the resting activity of VIIIth nerve fibres. Thus, a primary vestibular afferent responsive to a single canal was identified. Thermal stimulations greater than the ones usually used, applied to the other ampullae, did not modify the resting activity of this vestibular fibre. In this way, it was possible to rule out thermal stimulus spread to additional semicircular canals.

Click and diapason (440 Hz) stimulations at variable intensity were used to study the binocular acoustic influences on MGB units. Click stimuli were obtained by applying 0.1 msec pulses delivered from a 160–161–162 Tektronix waveform generator appropriately amplified and connected to a loudspeaker. This latter stimulus was preferred because of the similarity between its components and the ones commonly occurring in most natural sounds. In addition, clicks produce widespread activity throughout the acoustic pathway and higher auditory neurons while pure tones do not (Aitkin & Dunlop 1968, 1969).

The thermal stimulation of the single semicircular canals could be performed in the absence of auditory stimuli as the animals were isolated acoustically. The recorded sites in the

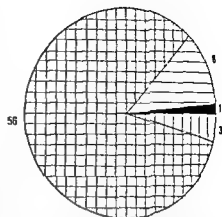


Fig. 2. The chart illustrates the responsiveness of the 56 units recorded in MGB. Black area: non responsive units. Vertical bars: acoustically affected units. Horizontal bars: units driven only by vestibular inputs. Cross-hatched area: units influenced by both types of stimulation.

brain stem were marked by discrete electrolytic lesions. At the conclusion of each experiment, the animal was given a lethal dose of anaesthetic, the brain stem was removed, fixed in Carnoy solution, embedded in paraffin, sectioned (12  $\mu\text{m}$ ) serially and stained according to the Nissl method for the localization of the microelectrode tip (Fig. 1).

## RESULTS

In the present experiments, 90 units have been recorded. 68 were localized in the medial geniculate bodies, 16 in the *colliculi inferioris* and 6 in the *brachia colliculi inferioris*.

Regarding the units recorded within the MGB, their histological localization was determined following the anatomical classification of the guinea pig thalamus by Gerebztad (1950). Two units were recorded from the *nucleus dorsalis*, 51 from the *nucleus profundus* and 15 from the *nucleus ventralis*. However, the MGB has been dealt with as one entity since response types were not characteristically unique for each of these nuclei (Liedgren et al., 1976).

In resting conditions, 23 out of 68 MGB units (33.8%) exhibited tonic activity with discharge frequency ranging from 10 to 25/sec.

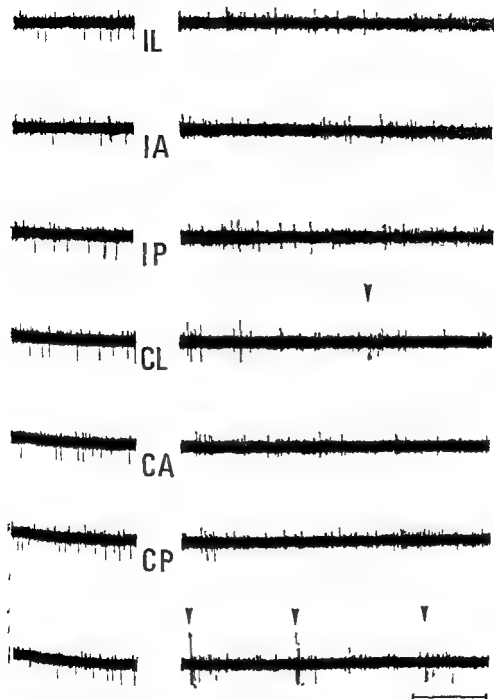


Fig. 3. Records taken from left medial geniculate body following vestibular and acoustic stimulations. Unit no. 64. The left column shows the unitary discharge in resting conditions; the right one, during stimulation. Note in the first six strips total blocking of unitary discharge following thermal stimulation of the six single semicircular canals. In the last strip the same unit was tested with

click (arrows) stimuli. Note in CL a burst of activity induced by click during vestibular inhibition. Abbreviations: IL, ipsilateral lateral; IA, ipsilateral anterior; IP, ipsilateral posterior; CL, contralateral lateral; CA, contralateral anterior; CP, contralateral posterior, canals. Calibration: 25 mm/sec.

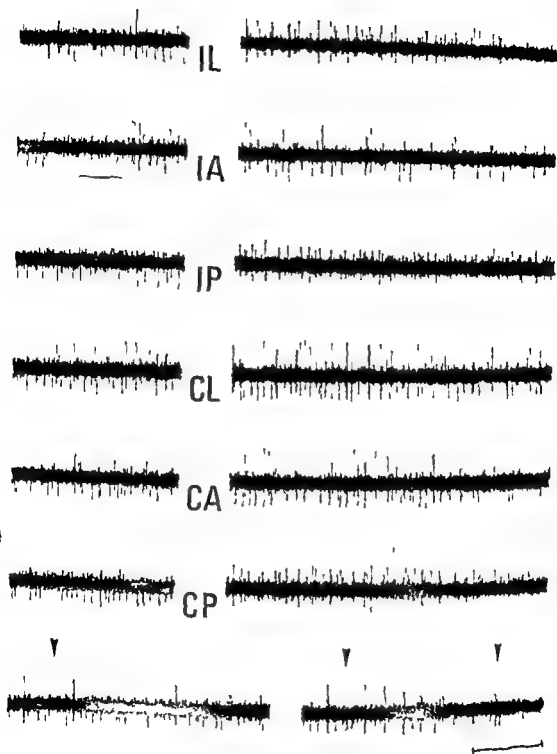


Fig 4 Unitary recordings taken from right medial geniculate body (Unit no. 59). In the first six strips, effects of thermal stimulation applied to the single semicircular canals: control on the left, responses on the right side. Following *IL*, *IP* and *CP* separate stimulations, the unitary discharge was inhibited and recruitment of new units was

observed. A slight decrease in frequency rate is shown. *IA*, *CL* and *CA* stimulations were ineffective (as shown by arrows in the last strip, delivered to the unit, enhanced the discharge rate. Abbreviations and stimulation as in Fig. 3).

Table 1. Number of MGB units influenced by thermal stimulation of individual semicircular canals

Semicircular canals	Responsive units
	5
	6
	11
	18
	9
	15
Total	64

Forty-four (64.7%) units showed tonic activity with irregular discharge frequency. Only one unit (1.5%) presented rhythmic discharge with a frequency of 30/sec. The unitary polarity was mostly negative-positive, although at times some positive-negative spikes were recorded. In order to minimize possible changes in firing rate due to the variable excitability of the MGB neurons (Wepstein, 1966), the effects of the stimulation were compared with those immediately preceding unitary distribution. Furthermore, the unitary waveform and discharge were repeatedly observed at high speed sweep, throughout the whole period of stimulation in order to exclude possible frequency modifications due to changes in electrode position and to recording of other cells.

Sixty-four of the 68 recorded units were affected by vestibular stimulation and 56 of these units also responded to acoustic stimuli. Three units were driven by the auditory stimulation only and one unit was completely unresponsive (Fig. 2).

All the acoustically influenced units, irrespective of their histological localization, displayed a brief excitation represented by bursts of 8-18 spikes which followed every single click (Figs. 3, 4). Marked variability in excitatory response frequency occurred, independently of stimulus intensities (Aitkin et al. 1966). The same pattern of modification was also observed when clicks were applied during vestibular thermal stimulation (Fig. 3, L).

All the vestibular affected units were recorded from the nuclei profundus and ventralis. Thermal stimulation of individual semicircular canals of both labyrinths affected 42 units, while 22 were influenced by only one labyrinth (14 contra and 8 ipsilaterally). Most units were driven by more canals, as shown in Table 1, from which the convergence of more ampullary inputs on the same unit can be appreciated. Vestibular peripheral stimulation modified the discharge pattern of MGB units independently of the stimulated canal and no preponderance of effectiveness among canals was observed. Table II shows the effects induced by stimulation of ipsi- and contralateral single semicircular canals on the activity of the MGB units recorded. Modifications of unitary discharge appeared after 2-4 sec from stimulus onset and disappeared when the stimulus was over; they consisted of inhibition (or disfacilitation) or excitation (or disinhibition). A decrease in discharge rate to 5-10/sec (Fig. 4, 1A) was the most frequently encountered modification, total blocking of unitary discharge (Fig. 3) was also observed. The excitatory response consisted of an increase in discharge frequency up to 20-60/sec. Occasionally recruitment of new units could be recorded (Fig. 4, 1L, 1P, 1CP) and some tonic units could exhibit rhythmic activity following labyrinthine stimulation.

From the analysis of 22 units recorded in the inferior colliculus and its brachium, 33% excitatory effects and 15% inhibitory influences were obtained, following single canal stimulations. Bilaterality of labyrinthine projections was also encountered at these levels (Fig. 5).

## DISCUSSION

In previous works, convergence of vestibular inputs on several brain stem units localized in the lateral vestibular nucleus, paramedian pontine reticular formation, oculomotor nucleus, nystagmogenic cortical area has been demonstrated in the guinea pig (Desole & Palestini, 1969; Manni & Desole, 1966; Pal-

Table II Pattern of response of MGB units to thermal stimulation of single semicircular canals

Canals	Excitation	Inhibition	No influence	Total influenced units
IL	10	21	33	64
IA	10	12	42	64
IP	11	20	33	64
CL	13	21	30	64
CA	16	16	32	64
CP	11	24	29	64
Totals	71	114	199	

strini & Desole, 1974, Petrosini et al, 1975, Troiani et al, 1976). The purpose of the present work was to analyse, in single units localized in the medial geniculate body, convergence of labyrinthine inputs from single semicircular canals and to investigate acoustical and vestibular convergence. Thermal stimulation applied to individual osseous ampullae has been proven to be selective, as demonstrated in the Methods, and advantageous for the study of afferences from single canals on single neurons. An analysis of unitary discharge of MGB neurons in the guinea pig has never been undertaken, notwithstanding the anatomic functional indications of Gerebtzoff (1950) on the arrival on this thalamic nucleus of vestibular fibres on their way to the cortex.

According to our results the medial geniculate body is reached by vestibular and acoustic afferences in the territory of the *nuclei ventralis* and *profundus*, while units localized in the *nucleus dorsalis* were not influenced by vestibular stimulation. This is in agreement with the classification of Gerebtzoff (1950) who claimed that the *nucleus dorsalis* is reached by acoustic and optic fibres only. The patterns of unitary modification recorded in MGB following vestibular stimulation can be summarized as follows: (i) 94% of the units analysed were influenced by vestibular inputs, of which (ii) 62% responded to separate stimulation of both labyrinths and 32% to only one (mainly contralaterally), (iii) 72% of the re-

corded units were affected by inflows arising from several canals and 22% from one canal only, (iv) the MGB unitary responses following stimulation of individual semicircular canals of the ipsilateral labyrinth were quite similar to those obtained by stimulating the contralateral single ampullae.

On this basis it seems reasonable to assume a wide vestibular projection on MGB. This result is in agreement with recent electrophysiological studies in cat and monkey which have demonstrated that vestibular thalamic projections are directed particularly to the ventro basal complex and to the posterior nuclear group, including MGB (Troiani et al, 1972, Deecke et al, 1974, Liedgren & Schwarz 1976, Liedgren et al 1976, Rammond et al, 1974, Sans et al, 1970, Wepfer 1966). The responsiveness of MGB neurons to both labyrinths, indicates a bilateral area of vestibular inflow, this result gains support from Odqvist et al (1973) who demonstrated on the bilateral cortical representation of the vestibular nerve in the guinea pig. The discharge modifications induced on the MGB unit by inputs from single ampullae indicate the existence of convergence of lar volleys on these neurons. Therefore MGB units under the influence of inputs from both vertical and horizontal canals, this thalamic nucleus can be assumed to be a projection structure to other subcortical and cortical centres of information on head movement and position (Manni, 1952).

The MGB unitary discharge pattern was modified by vestibular peripheral stimulation with either excitation or inhibition, the latter being the most frequently observed. Considering that the same MGB units were influenced by both acoustic and vestibular inputs, an attempt to explain the vestibular inhibitory response leads us to suppose a inhibitory action of Golgi II interneurons (Verossy & Kiss 1976a, 1976b), as demonstrated with intracellular recordings by other authors following acoustic stimulation (Aitkin & Pop, 1968, 1969, Etholm, 1976). For

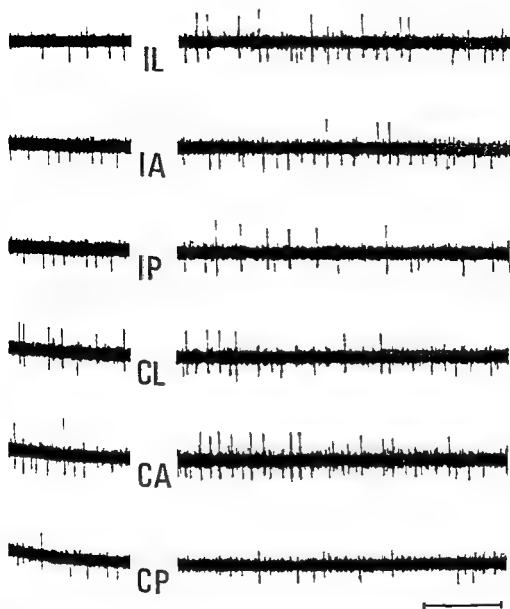


Fig. 3. Unit no. 5 recorded from left *brachium coli cul* ferens. The left column depicts the resting unitary discharge; the right one the response to thermal stimulation of the left single semicircular canal. Four canal

(IL, IA, CL, CA) stimulation increased the discharge rate while both posterior canals were ineffective. Abbreviations and calibration as in Fig. 3.

units recorded in the inferior colliculus and its *brachium* were driven by thermal labyrinthine stimulation mostly with an increase in discharge frequency. Consequently the inhibitory observed in MGB ought to be generated by intrinsic circuits. MGB unitary responses to our acoustic

stimulation always given binaurally consisted in agreement with previous investigations (Atkin et al. 1966; Atkin & Dunlop 1968) of excitatory effects. Convergence of acoustic and vestibular inputs on the same MGB unit is thus claimed.

Speculations on the possible role of this



thalamic nucleus regarding the mechanism of head orientation need more detailed physiological studies and will be the aim of further investigations

## ACKNOWLEDGEMENTS

The authors express their gratitude to Miss B. Zannoni for her excellent histological and technical assistance. The help of the medical students B. Croni and R. Isabella during the experimentation has been duly appreciated. Mr R. Mancinelli is to be thanked for his technical aid.

## ZUSAMMENFASSUNG

In den curarisierten Meerschweinchen wurden 68 Neurone des Corpus Geniculatum Mediale (CGM) mit vestibulären und akustischen Reizen untersucht. Die einzelnen Bogengänge wurden termisch gereizt. Konvergenz der vestibulären und akustischen Afferenzen an denselben Einheiten des CGM wurden registriert. Der Reizung der Bogengänge folgend, wurden Hemmung und Aktivierung der Einheiten aufgezeichnet, wobei die Hemmung vorherrschend war, während bei akustischer Reizung („Klick“) Salven neuronaler Aktivität verzeichnet wurden. Es wird die Beteiligung des CGM in die vestibulo-akustische Integration postuliert.

## REFERENCES

- Atkin, L. M. & Dunlop, C. W. 1968 Interplay of excitation and inhibition in the cat medial geniculate body. *J Neurophysiol* 31, 44.
- 1969 Inhibition in the medial geniculate body of the cat. *Exp Brain Res* 7, 28.
- Atkin, L. M., Dunlop, C. W. & Webster, W. R. 1966 Click evoked response patterns of single units in the medial geniculate body of the cat. *J Neurophysiol* 29, 109.
- Copack, P., Dafny, N. & Gilman, S. 1972 Neurophysiological evidence of vestibular projections to thalamus, basal ganglia and cerebral cortex. In *Corticothalamic Projections and Sensorimotor Activities* (ed T. L. Fry, E. Rinvik & M. D. Yahr) pp. 309–339. Raven Press, New York.
- Deecke, L., Schwarz, D. W. F. & Fredrickson, J. M. 1973 The vestibular thalamus in the Rhesus monkey. *Adv Otorhinolaryngol* 19, 210.
- 1974 Nucleus ventroposterior inferior (VPI) as the vestibular thalamic relay in the Rhesus monkey. I. Field potential investigation. *Exp Brain Res* 20, 88.
- Desole, C. & Palestini, E. A. 1969 Responses of vestibular units to stimulation of individual semicircular canals. *Exp Neurol* 24, 310.
- Etholm, B. 1976 Activity of single medial geniculate units in response to single and double clicks. *Acta Otolaryngol* (Stockh) 81, 91.
- Flourens, P. 1842 *Recherches expérimentales sur les propriétés et les fonctions du système nerveux animal, vertébrés*. 2nd edn. J. B. Baillière, Paris.
- Fredrickson, J. M., Kornhuber, H. H. & Schwarz, D. W. F. 1974 Cortical projection of the vestibular system. In *Handbook of Sensory Physiology* vol. 11, 1. *Vestibular System* (ed H. H. Kornhuber), pp. 24–44. Springer, Berlin.
- Gerebtzoff, M. A. 1950 Connexions et organisation du thalamus. *Arch Int Neurol* 1, 1.
- Gernandt, H. E. 1950 Midbrain activity in response to vestibular stimulation. *Acta Physiol Scand* 21, 7.
- Hassler, R. 1948 Forels Haubenfaszikel als vestibuläre Empfindungsbahn mit Bemerkungen über einige andere sekundäre Bahnen des Vestibulars und Trigemini. *Arch Psychiatr Nervenkr* 130, 23.
- 1964 Spezifische und unspezifische Systeme im menschlichen Zwischenhirn. In *Lectures on the brain cephalon* (ed W. Bargmann & J. W. Schade) pp. 1–12. Elsevier, Amsterdam.
- 1972 Hexapartition of inputs as a primary role of the vestibular system. In *Neurophysiology of the vestibular system* (ed J. M. Fredrickson & D. W. F. Schwarz) pp. 1–12. Raven Press, New York.
- of vestibular afferents in somatosensory nuclei of the squirrel monkey (*Saimia sciurina*). *Neurophysiol* 39, 601.
- Lorente de No, R. 1931 Die Augenmuskelfähre bei Kanarienvögeln und ihre Grundlagen. *Ergeb Physiol* 2, 73.
- Majorossy, K. & Kiss, A. 1976a Specific patterns of neuron arrangement and of synaptic articulation in the medial geniculate body. *Exp Brain Res* 26, 1.
- 1976b Types of interneurons and their participation in the neuronal network of the medial geniculate body. *Exp Brain Res* 26, 19.
- Mancinelli, R. 1975 A simple technique for thermal stimulation of semicircular canals. *Arch Ital Biol* 111, 26.
- Manni, E. 1952 Effetto di lesioni di alcune strutture mesencefaliche e talamiche sul tono muscolare e sulla riflessa vestibolare. *Arch Fisiol* 52, 250.
- Manni, E. & Desole, C. 1966 Responses of ocular units to stimulation of single semicircular canals. *J Neurol* 15, 206.
- Mickle, W. A. & Ades, H. W. 1954 Rostral projection pathway of the vestibular system. *Am J Physiol* 17, 243.
- Ödkvist, L. M., Rubin, A. M., Schwarz, D. W. F. & Fredrickson, J. M. 1973 Vestibular and auditory cortical projection in the guinea pig. *Exp Brain Res* 27, 279.
- 1974 Relationship of vestibular afferents to the cerebral cortex. In *Neurophysiology of the vestibular system* (ed J. M. Fredrickson & D. W. F. Schwarz) pp. 1–12. Raven Press, New York.
- Petrosini, L., Tironi, D. & Schwarz, D. W. F. 1974 Convergence of afferent impulses from individual semicircular canals on pontine reticular units. *Arch Ital Biol* 111, 1.

- J Sans A & Marty J 1974 Projections lam ques des noyaux vestibulaires Etude histologique chat *Exp Brain Res* 20 273
- A Raymond J & Marty R 1970 Réponses thalamiques corticales à la stimulation électrique du nerf vestibulaire chez le chat *Exp Brain Res* 10 265
- egel E A, Szchily E J & Gildenberg P C 1965 Vestibular response in midbrain, thalamus and basal ganglia *Arch Neurol* 12 258
- D Petrosini L & Zannoni B 1976 Relations of single semicircular canals in the pontine reticular formation *Arch Ital Biol* 114 357
- Wepsc J J 1966 Multimodal sensory activation of cells in the magnocellular medial geniculate nucleus *Exp Neurol* 15 299

Diana Troiani M D  
Istituto di Fisiologia Umana  
Università Cattolica S. Cuore  
Via della Pigna Sacchetti 644  
00168 Roma  
Italy

# ACTOMYOSIN ATPase ACTIVITY OF HUMAN LARYNGEAL MUSCLES

E Teig, H A. Dahl and H Thorkelsen

*From the Ear Nose and Throat Department Rikshospitalet The Norwegian College of Physical Education and Sport and The Anatomical Institute University of Oslo Norway*

(Received March 2, 1977)

**Abstract** The muscles from seven human larynxes removed by laryngectomy have been examined for actomyosin ATPase by histochemical methods. The various muscles contained a mixture of ATPase low (type I) and ATPase high (type II) muscle fibres. The thyroarytenoid muscle had the highest proportion of type II fibres (65%) and the posterior cricoarytenoid muscle had the highest proportion of type I fibres (67%). The other laryngeal muscles had intermediate values. All human laryngeal muscles had a higher percentage of type I fibres than the corresponding muscles in animals so far examined, a finding which may be related to the development of speech.

There is a close correlation between the contraction speed of a muscle and its activity of actomyosin ATPase (Bárány, 1967). In most mammalian muscles two main types of muscle fibres can be identified, one with low actomyosin ATPase activity (type I) and the other with high actomyosin ATPase activity (type II) (Guth & Samaha, 1969; Brooke & Kaiser, 1970).

In a combined physiological-histochemical study in the cat it has been shown that the motor units consisting of type II muscle fibres have a fast contraction speed and those consisting of type I fibres have a low contraction speed (Burke et al., 1973).

The contraction speed of the laryngeal muscles has been the subject of interest to many investigators. While the thyroarytenoid muscle in most species appears to be a fast muscle (Mårtensson & Skoglund, 1964; Hirose

et al., 1969; Hast, 1969) or even, as in the rabbit, an extremely fast muscle (Hall-Craggs, 1968) there appear to be certain species differences with other laryngeal muscles. The posterior cricoarytenoid muscle is fast in the cat (Hirose et al., 1969), but slow in the dog (Mårtensson & Skoglund, 1964; Hast, 1969). The cricothyroid muscle is fast in the squirrel monkey (Hast, 1969) and in the rabbit (Hall-Craggs, 1968), but slow in the gibbon, the macaque (Hast, 1969), in the dog (Mårtensson & Skoglund, 1964; Hast, 1966) and in the cat (Hirose et al., 1969).

There is also considerable interspecies variation in the proportion of type I and type II fibres in the various laryngeal muscles in animal species examined so far (Asmusen & Wohlrab, 1972; Sahgal & Hast, 1974).

With the development of speech it is likely that the laryngeal muscles in man have evolved differently from those of the common laboratory animals. The aim of the present investigation was to see to what extent this functional difference is reflected in the proportions of type I and type II muscle fibres.

## MATERIALS AND METHODS

Intrinsic laryngeal muscles were dissected from the healthy side of human larynxes obtained during laryngectomy on 7 males with

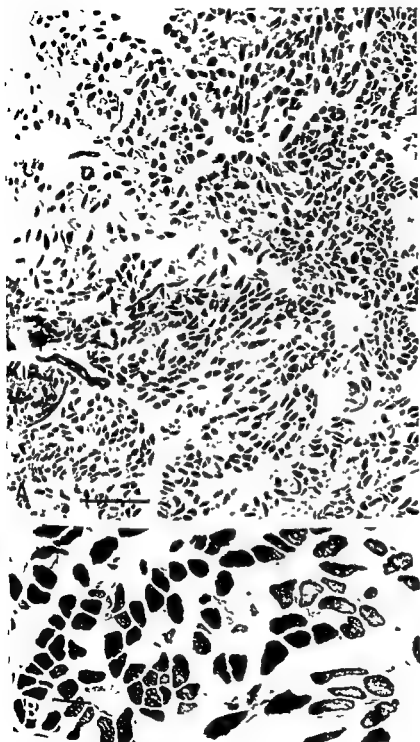


Fig 1A B Human thyroarytenoid muscle Patient treated with 60 Gy ( $\approx 6000$  rad) cobalt 3 months preoperatively 16  $\mu$ m section incubated for ATPase at pH 9.4 after preincubation in buffer pH 10.3 with 50 mM  $\text{CaCl}_2$  and buffer pH 4.6 Type I muscle fibres light Type II muscle fibres dark Scale 500  $\mu$ m (A) and 100  $\mu$ m (B)

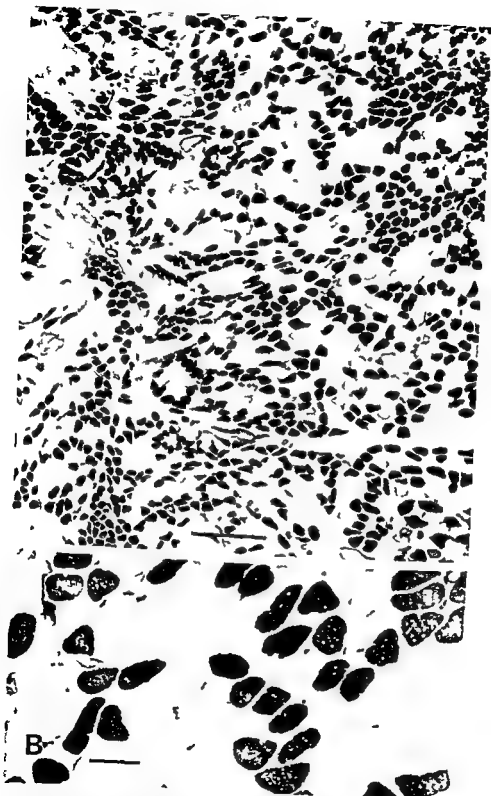


Fig. 2 A, B Human cricothyroid muscle. Patient treated with conventional radiation (300 kV) 4 000 R total skin dose and 180 mg Bleomycin 1 year preoperatively. Incubated on and preincubated on conditions as in Fig. 1 at 500  $\mu$ m (A) and 100  $\mu$ m (B).

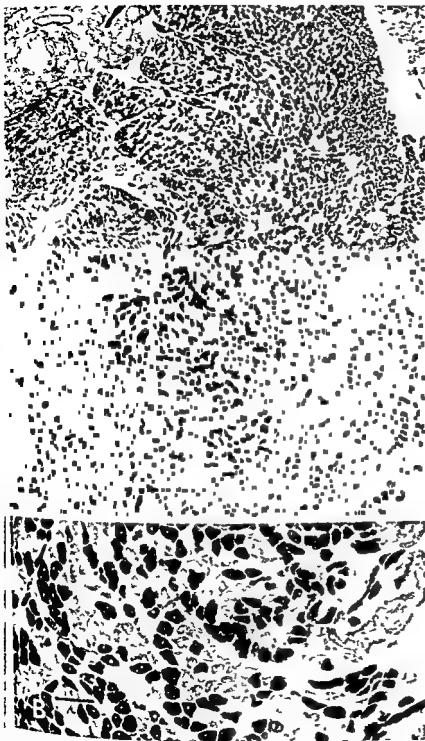


Fig 3A B Human interarytenoid muscle. Primary laryngectomy. Incubation and preincubation conditions as in Fig 1. Scale 500  $\mu$ m (A) and 100  $\mu$ m (B).

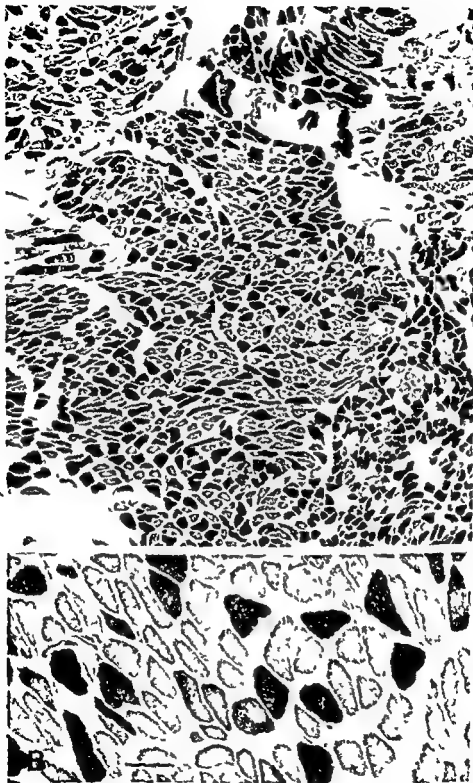
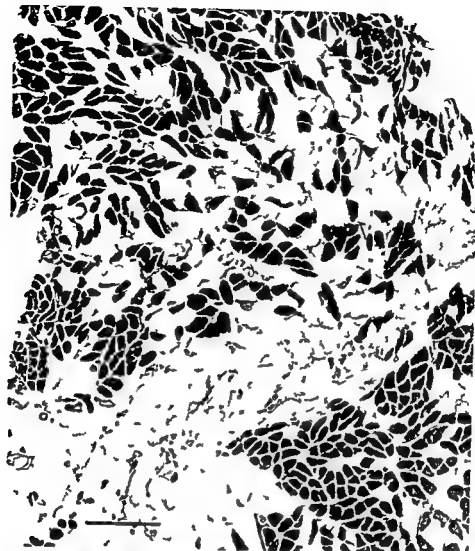


Fig 4A B Human lateral cricoarytenoid muscle from patient treated with conventional radiation (300 kV) 4200 R total skin dose and 180 mg Bleomycin 8 months pre-operatively. Conditions otherwise as in Fig 1 except the alkaline buffer contained 100 mM  $\text{CaCl}_2$  instead







*Fig. 5A, B* Type grouping of muscle fibres in human posterior cricoarytenoid muscle. Same patient and histochemical treatment as in Fig. 2.

Table I

Muscle	% type I ± S D	% type II ± S D
Thyroarytenoid	35 ± 11.6	65 ± 11.6 n=7
Lateral cricoarytenoid	40 ± 5.2	60 ± 5.2 n=6
Interarytenoid	46 ± 8.7	54 ± 8.7 n=5
Cricothyroid	47 ± 6.1	53 ± 6.1 n=7
Posterior cricoarytenoid	67 ± 8.6	33 ± 8.6 n=7

cles. Acid preincubation at different pH values was attempted in order to identify subgroups of the type II fibres (Brooke & Kaiser, 1974). Inactivation of the ATPase activity of most type II fibres took place gradually in the range pH 4.6-4.2. It was therefore not possible to tell with certainty which of the muscle fibres corresponded to the different subgroups of the type II fibres.

## DISCUSSION

The present results show that there is a great variation from person to person in the proportion of type I and type II fibres in the various laryngeal muscles. In this respect the laryngeal muscles resemble ordinary human skeletal muscles, which also show considerable interpersonal variation (Johnson et al., 1973).

The high percentage of type II muscle fibres in the thyroarytenoid muscle is surprising considering that 70-80% of the fibres in the medial two-thirds of this muscle have been found to have multiple motor end plates (Rossi & Cortesma 1965). In lower vertebrates multiply innervated muscle fibres have been shown to be of slow non twitch type (Kuffler & Vaughan Williams, 1953). In the cat, multiply innervated muscle fibres have been found in the external eye muscles (Hess & Pilar, 1963) and in the middle ear muscles (Fernand & Hess 1969). From existing evidence they seem to be of both slow non twitch and of slow twitch type (Hess & Pilar, 1963, Hess, 1970, Bach y Rita & Ito, 1966, Lennnerstrand 1974, Browne, 1976).

According to Hall Craggs (1968) the thy-

roarytenoid muscle of the rabbit is extremely fast, and although interspecies variations may exist, it is still hard to account for the high percentage of multiply innervated muscle fibres in the thyroarytenoid muscle. More research on this point is necessary to clarify the matter.

It is not possible from the proportion of type I and type II fibres alone to tell whether a muscle as a whole is fast or slow. However, by comparison with other muscles, e.g. the cat tensor tympani which is a fast muscle (Teig, 1972) and which consists of 40% type I and 60% type II muscle fibres (Teig & Dahl, 1972), it seems probable that the thyroarytenoid muscle in man is a fast muscle.

In the rabbit Asmussen & Wohlrab (1972) found the thyroarytenoid muscle to consist exclusively of type II fibres, while the cricothyroid muscle consisted of 65% type II and 35% type I fibres, and the posterior cricoarytenoid muscle of 60% type II and 40% type I fibres. Comparable ATPase activity was found in muscle homogenates from rabbit cricothyroid and thyroarytenoid muscles (Srovy & Gutmann, 1971). In the rhesus monkey Sahgal & Hast (1974) found 81% type II and 19% type I fibres in the thyroarytenoid, 70% type II and 30% type I in the cricothyroid and 88% type II and 12% type I in the lateral cricoarytenoid muscle.

The present results show that the percentage of type I muscle fibres in all the laryngeal muscles in man is much higher than in the animals so far examined. This must be kept in mind when data from man are related to data from experimental animals. Since type I muscle fibres at least in ordinary skeletal muscles are slow and more resistant to fatigue than type II muscle fibres (Burke et al., 1973) the high percentage of type I muscle fibres in human laryngeal muscles might be related to the development of speech with demand for frequent and longlasting activity of these muscles.

The muscles with the highest proportion of type II muscle fibres, the thyroarytenoid,

the lateral cricoarytenoid and the interarytenoid, all contribute to the sphincter function of the larynx. The need for fast action in this protective mechanism which is the phylogenetically oldest and most important function of the larynx (Negus, 1949), is evident. The cricothyroid muscle, whose main action is to produce tension in the vocal cords, and which is the most important muscle for the variation of pitch (Koyama et al., 1971), has a higher proportion of type I muscle fibres. Again the higher proportion of type I fibres in this muscle in man might reflect a need for endurance and fatigue resistance in voice production. The sole abductor of the larynx, the posterior cricoarytenoid, which is active during the inspiratory phase of respiration (Faaborg-Andersen, 1957) has a higher proportion of oxidative enzymes than the other laryngeal muscles (Hefter, 1967, Hanson & Lotz, 1973) and was found to have the highest proportion of type I fibres in the present investigation. This constellation is probably related to the continuously rhythmic action of this muscle during respiration.

When the recurrent nerve of experimental animals is stimulated with supramaximal stimulus amplitude, a low stimulus frequency induces abduction of the vocal cords, whereas higher stimulus frequency leads to an increasing adduction (Ohyama et al., 1972). This has also been observed in the human larynx (Kotby & Haugen, 1970).

The present findings offer a possible explanation of this. The slow muscle fibres are brought into tetanic contraction and thus exercise their full force at a low stimulus frequency while the fast fibres need a considerably higher stimulus frequency to reach full tetanic tension. A low stimulus frequency would therefore be more effective to the posterior cricoarytenoid muscle than to the other muscles, and a high stimulus frequency, giving tetanic contraction of all muscle fibres, would lead to adduction since there is an overweight of adductor muscles in the larynx (Pressman & Kelemen, 1955).

"Grouping" of type I and type II fibres in muscle is assumed to be a result of muscular degeneration where denervated muscle fibres are reinnervated by resprouting from adjacent intact motor (e.g. Edstrom & Kugelberg 1969). Laryngeal paresis with impaired abductor function is relatively common in horses ("roarers") (C 1972) found type "grouping" of fibres in the posterior cricoarytenoid muscle in a few apparently normal horses. This was interpreted as indicating a tendency to spontaneous degeneration of this muscle, and it was guessed that the affected horses later might have become clinical "roarers".

The type "grouping" in the posterior cricoarytenoid muscle in one of our patients is an unexpected finding since the patient had no clinical signs of laryngeal paresis. The posterior cricoarytenoid muscle is more exposed to mechanical trauma such as endoscopy or even possibly swallowing of lumps of food, and this might be an explanation for the selective affection of this muscle in our patient. The finding also lends support to the much disputed Semons' law of the greater vulnerability of this sole abductor muscle of the larynx.

## ZUSAMMENFASSUNG

Die Muskeln sieben Kehlköpfe, die durch Entfernung von Kehlkopfknorpel und Kehlkopfknorpel entfernt worden waren, wurden auf Actomyosin mit histochemischen Methoden untersucht. In diesen Muskeln enthielten ein Gemisch von ATP-schwachen (Typ I) und ATP-starken (Typ II) Fasern. Der grösste Anteil Typ II Fasern (67%) in Muskulus thyroarytenoideus der grösste Anteil I Fasern (67%) in Muskulus cricoarytenoideus zu. Die übrigen laryngealen Muskeln zeigten durchschnittliche Werte. Die laryngealen Muskeln des Menschen zeigten einen höheren Anteil von Typ I Fasern als entsprechenden Muskeln der Tiere. Ein Befund der Entwicklung der Sprache möglicherweise z. hängt.

## REFERENCES

- Asmussen G & Wohlrab F 1972 Enzymhistochemische Untersuchungen an der Kehlkopf- und Kehlkopfknorpelmuskulatur des Kanarienvogels. *Z. f. Anat. Anat. Forsch.* 85: 181
- Bach y Rita P & Ito F 1966 In vivo studies

- and slow muscle fibers in cat extraocular muscles *J Gen Physiol* 49 1177
- rany M 1967 ATPase activity of myosin correlated with speed of muscle shortening *J Gen Physiol* 50 Suppl 197
- M H & Kaiser K 1970 Muscle fiber types. How many and what kind? *Arch Neurol* 23 369
- 1974 The use and abuse of muscle histochemistry *Ann NY Acad Sci* 228 121
- owze J S 1976 The contractile properties of slow muscle fibres in sheep extraocular muscle *J Physiol (Lond)* 254 535
- R E Levine D N Tsarris P & Zajac F E 1973 Physiological types of histochemical profiles in motor units of the cat gastrocnemius *J Physiol (Lond)* 234 723
- lstrom L & Kugelberg E 1969 Histochemical mapping of motor units in experimentally re-innervated skeletal muscle *Experientia* 25 1044
- mand V S V & Hess A 1969 The occurrence structure and innervation of slow and twitch muscle fibres in the tensor tympani and stapedius of the cat *J Physiol (Lond)* 200 547
- aborg Andersen K 1957 Electromyographic investigation of intrinsic laryngeal muscles in humans *Acta Physiol Scand* 41 Suppl 140
- man H M 1972 Histochemical observation on laryngeal skeletal muscle fibres in normal horses *Equine Vet J* 4 144
- uth L & Samaha F J 1969 Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle *Exp Neurol* 25 138
- uth L & Samaha F J 1970 Procedure for the histochemical demonstration of actomyosin ATPase *Exp Neurol* 28 365
- ull-Craggs E C B 1968 The contraction times and enzyme activity of two rabbit laryngeal muscles *J Anat* 102 241
- anson J & Lotz P 1973 Der Energiestoffwechsel der menschlichen Kehlkopfmuskulatur aus der Sicht der LDH Iso-enzymverteilung *Mischr Ohren Heilkd* 107 218
- ast M H 1966 Mechanical properties of the cricothyroid muscle *Laryngoscope* 76 537
- 1967 The respiratory muscle of the larynx *Ann Otol Rhinol Laryngol* 76 489
- 1969 The primate larynx - a comparative physiological study of intrinsic muscles *Acta Otolaryngol (Stockh)* 67 84
- Hefer E 1967 Enzymhistochemische Untersuchungen an Kehlkopfmuskeln des Menschen *Arch Ohren Nasen Kehlkopfheilkd* 288 504
- Hess A 1970 Vertebrate slow muscle fibers *Physiol Rev* 50 40
- Hess A & Pilar G 1963 Slow fibres in the extraocular muscles of the cat *J Physiol (Lond)* 169 780
- Hirose H Ushijima T Kobayashi T & Sawashima M 1969 An experimental study of the contraction properties of the laryngeal muscles in the cat *Ann Otol Rhinol Laryngol* 78 297
- Johnson M A Polgar J Weightman B & Appleton D 1973 Data on the distribution of fibre types in thirty six human muscles. An autopsy study *J Neurol Sci* 18 111
- Kotby M N & Haugen L K 1970 Attempts at evaluation of the function of various laryngeal muscles in the light of muscle and nerve stimulation experiments in man *Acta Otolaryngol (Stockh)* 70 419
- Koyama T Harvey J E & Ogura J H 1971 Mechanics of voice production II Regulation of pitch *Laryngoscope* 81 45
- Kuffler S W & Vaughan Williams E M 1953 Properties of the slow skeletal muscle fibres of the frog *J Physiol (Lond)* 121 318
- Lennerstrand G 1974 Electrical activity and isometric tension in motor units of the cat's inferior oblique muscle *Acta Physiol Scand* 91 458
- Mårtensson A & Skoglund C R 1964 Contraction properties of intrinsic laryngeal muscles *Acta Physiol Scand* 60 381
- Negus V E 1949 *The Comparative Anatomy and Physiology of the Larynx* William Heineman Medical Books London
- Ohyama M Ueda N Harvey J E Mogi G & Ogura J H 1972 Electrophysiological study of re-innervated laryngeal motor units *Laryngoscope* 82 237
- Pressman J J & Kelemen G 1955 Physiology of the larynx *Physiol Rev* 35 506
- Rossi G & Cortesina G 1965 Morphological study of the laryngeal muscles in man *Acta Otolaryngol (Stockh)* 59 576
- Sahgal V & Hast M H 1974 Histochemistry of primate laryngeal muscles *Acta Otolaryngol (Stockh)* 78 277
- Syrovy L & Gutman E 1971 ATPase activity of two rabbit laryngeal muscles *Experientia* 27 248
- Teig E & Dahl H A 1972 Actomyosin ATPase activity of middle ear muscles in the cat *Histochemie* 29 1
- Teig E 1972 Force and contraction velocity of the middle ear muscles in the cat and the rabbit *Acta Physiol Scand* 84 1

E Teig MD  
ENT Dept  
Rikshospitalet  
Oslo  
Norge

# QUANTITATION OF HUMAN GRANULOCYTE PROTEASE INHIBITORS IN NON PURULENT BRONCHIAL LAVAGE FLUIDS

H Tegner

*From the Departments of Clinical Chemistry and Otorhinolaryngology  
Malmö General Hospital University of Lund Sweden*

(Received April 22 1977)

**Abstract** The predominant inhibitors of granulocyte proteases in plasma ( $\alpha_1$  antitrypsin  $\alpha_1$  antichymotrypsin and  $\alpha_2$ -macroglobulin) were quantitated in unconcentrated bronchial lavage fluids obtained from non infected individuals together with the acid stable low molecular weight inhibitor with activity against granulocyte elastolytic and chymotrypsin like enzymes. This latter inhibitor accounted for about 90% of the total molar concentration of granulocyte protease inhibitors in the bronchial lavage fluids. The remaining 10% consisted mostly of  $\alpha_1$  antitrypsin and  $\alpha_1$  antichymotrypsin. About 85% of the bronchial inhibitor was in a free form with preserved enzyme reactivity. The remaining 15% of the immunoreactive bronchial inhibitor exhibited a molecular size indicating complexation with enzymes. The major portion of  $\alpha_1$  antitrypsin and  $\alpha_1$  antichymotrypsin showed electrophoretic mobilities and molecular sizes similar to the native proteins but had no enzyme reactivity.

Normal bronchial secretion is difficult to obtain from man, hence most earlier studies have been performed on purulent expectorates (Ryley & Brogan, 1973; Lebas et al., 1976). Specific immunochemical techniques demonstrate the presence of most serum proteins in such secretions (Biserte et al., 1963; Dennis et al., 1964) and albumin and IgA are thought to be the predominant plasma proteins (Anzai et al., 1963; Ibayashi et al., 1963; Dennis et al., 1964; Masson et al., 1965). The major inhibitors of proteolytic enzymes in plasma such as  $\alpha_1$ -antitrypsin,  $\alpha_1$  antichymotrypsin and  $\alpha_2$ -macroglobulin, have also been identified in bronchial secretions (Ryley & Brogan, 1973; Lebas et al., 1976).  $\alpha_1$ -Antitrypsin was shown

to be the inhibitor with the highest molar concentration as in plasma (Hochstrasser et al., 1972; Laurell & Jeppsson, 1975). This inhibitor inactivates the elastolytic, chymotrypsin like, and collagenolytic enzymes of granulocytes (Ohlsson & Olsson, 1973, 1974a, 1974b; Ohlsson, 1975; Venge et al., 1975) (Table I).  $\alpha_2$ -Macroglobulin also inhibits all known granulocyte proteolytic enzymes (Ohlsson & Olsson, 1973, 1974a; Ohlsson, 1975; Venge et al., 1975) but  $\alpha_1$ -chymotrypsin is specific for the chymotrypsin like granulocyte proteases (Ohlsson & Olsson, 1976) (Table I).

Bronchial secretions also contain an acid stable, low molecular weight inhibitor of proteolytic enzymes (Hochstrasser et al., 1972; Ohlsson et al., 1977) which is a strong inhibitor of elastolytic (Ohlsson & Tegner, 1976) as well as chymotrypsin like (Tegner et al., 1977) enzymes from granulocytes but not the collagenolytic enzyme (Ohlsson & Tegner, 1977) (Table I).

Granulocyte elastase was recently shown in a pilot study to rapidly destroy the mucocoe-

This investigation was supported by the Swedish Medical Research Council (project no. B77 17X-019104-00), the Medical Faculty University of Lund, the Swedish National Association against Heart and Chest Diseases, the Swedish Tobacco Company, the Swedish Society of Natural Sciences, Greta and Johan Kock's Foundation, the Österlund's Foundation and Murath's Foundation.

## Table 1 Inhibition specificity of the major protease inhibitors in human bronchial mucus

Inhibitor	Mol wt (D)	Inhibition of granulocyte proteases		
		Elastase	Collagenase	Chymotrypsin like cationic proteins
Antitrypsin	55 000	+	+	(+)
Macroglobulin	725 000	+	+	+
Chymotrypsin	69 000	+	+	+
Bronchial mucus	10 500	+	+	+

activity of respiratory epithelium in vitro (Tegner, 1977). The molar concentration of the inhibitors in bronchial lavage fluid may correlate with the relative importance of each inhibitor in the protection of the ciliated columnar epithelium of the respiratory tract from granulocyte proteases. The purpose of the present investigation was to quantitate the predominant inhibitors of granulocyte proteases in non-infected bronchial lavage fluids.

## MATERIAL AND METHODS

Bronchial lavage fluid was aspirated during bronchoscopy of 51 patients (35 males and 16 females). Their ages ranged from 35 to 78 years. The indications for bronchoscopy were usually prior hemoptysis, or suspected or proven malignancy. The patients were afebrile and exhibited no signs of respiratory tract infection or other lung disease at the time of bronchoscopy.

Under general anaesthesia a rigid bronchoscope was passed into one of the main stem bronchi without prior endotracheal intubation. 50 ml of sterile saline was instilled under sterile conditions and gently aspirated to prevent inadvertent damage to the mucous membrane. Blood-contaminated or purulent samples were discarded.

The debris and cells of the lavage fluids were separated by centrifugation at 3 000 g for 15 min within 2-4 hours after collection, and the supernatant was frozen and stored at -20°C until analysis which was performed

without prior concentration of the supernatant. For qualitative studies 0.5 ml of each lavage fluid specimen was pooled and concentrated to about 2 ml in an Amicon cell with a Diaflo UM 2 membrane at 0°C. This pool was used to determine the free or complexed inhibitor levels in the samples and to measure the total protease inhibiting capacity of the specimens.

**Special materials** Sephadex G 75 and G 150 were purchased from AB Pharmacia, Uppsala, Sweden, and agarose from Miles Seravac, Maidenhead, England.

**Antisera** Specific antisera against albumin,  $\alpha_1$ -antitrypsin,  $\alpha_1$ -antichymotrypsin, and  $\alpha_2$ -macroglobulin are available in our laboratory. Rabbit antiserum against the low molecular weight inhibitor from bronchial secretion was prepared as previously described (Ohlsson & Tegner, 1976).

**Granulocyte proteases** Granulocyte elastase was purified as described (Ohlsson & Olsson, 1974a) and chymotrypsin like cationic proteins were obtained from Dr Inge Olsson (Olsson & Venge, 1974).

**Reference proteins** A human serum pool of blood donors was used as a reference. The concentration of albumin was 46 g/l,  $\alpha_1$ -antitrypsin 1.35 g/l, antichymotrypsin 0.4 g/l and  $\alpha_2$ -macroglobulin 2.0 g/l.

**Molecular weights** The following molecular weights were used for the calculations of molar concentrations: albumin 66 500 (Rothschild et al., 1972),  $\alpha_1$ -antitrypsin 55 000 (Laurell & Jeppsson, 1975), antichymotrypsin 69 000

Table II Frequency and average concentrations of detectable quantities of selected proteolytic bronchial lavage fluid samples of 51 patients

Protein	n		Mg/litre Mean $\pm$ S D	Nmole/litre Mean $\pm$ S D
Albumin	51	100%	116.0 $\pm$ 146.4	1.744 $\pm$ 2.200
$\alpha_1$ -Antitrypsin	43	84%	5.5 $\pm$ 6.8	100 $\pm$ 123
Antichymotrypsin	30	59%	2.6 $\pm$ 2.4	38 $\pm$ 34
$\alpha_2$ Macroglobulin	21	41%	9.5 $\pm$ 10.6	13 $\pm$ 14
Bronch. protease inhibitor	39	76%	15.0 $\pm$ 11.5	1.427 $\pm$ 1.095

(Laurell & Jeppsson, 1975),  $\alpha_2$ -macroglobulin 725 000 (Laurell & Jeppsson, 1975), and the bronchial protease inhibitor 10 500 (Ohlsson et al., 1977)

#### Immunochemical techniques

Albumin and the plasma protease inhibitors were measured by electroimmuno assay (Laurell, 1972). The single radial immunodiffusion technique of Mancini et al. (1965) was used for quantitation of the bronchial inhibitor using purified inhibitor as a standard. Crossed immunoelectrophoresis was performed according to Ganrot (1972).

#### Gel filtration

Gel filtration of 0.5 ml of the concentrated pool of lavage fluids was performed on a Sephadex G-75 column (0.6  $\times$  100 cm) equilibrated with 0.04 M Tris-HCl buffer, pH 7.6, containing 0.2 M NaCl. A constant flow of 8 ml per hour was maintained and 0.5 ml fractions were collected. On the same column and under identical conditions the elution volumes were determined for Trasylol ( $M_r$  7000), cytochrome C ( $M_r$  12 500), myoglobin ( $M_r$  17 800), chymotrypsinogen A ( $M_r$  25 000) and egg albumin ( $M_r$  45 000). Gel filtration was also performed on a Sephadex G 150 column (1.5  $\times$  100 cm) equilibrated with the same buffer. This column was calibrated with myoglobin ( $M_r$  17 800), egg albumin ( $M_r$  45 000), bovine albumin ( $M_r$  69 000), haptoglobin type 1-1 ( $M_r$  98 000), and aldolase ( $M_r$  158 000). A linear function resulted when the logarithms of their molecular weights were plotted against their elution volumes. The fractions eluted after gel filtration

of pooled concentrated lavage fluids were analyzed for immunoreactive  $\alpha_1$ -antitrypsin, antichymotrypsin, and the bronchial inhibitor.

Reaction mixtures of bronchial lavage fluids and granulocyte elastase or chymotrypsin and cationic proteins corresponding to about and 100% saturation of the protease with capacity of the lavage fluids were incubated for 10 min at 27°C before analysis by crossed immunoelectrophoresis or gel filtration.

## RESULTS

About 1 to 2 ml of bronchial lavage fluid was aspirated from each patient. The fluid appeared colourless and the debris separated by centrifugation was scanty.

#### Quantitation of albumin, $\alpha_1$ -antitrypsin, $\alpha_1$ -antichymotrypsin, $\alpha_2$ -macroglobulin and bronchial inhibitor (Table II)

Albumin was detected in all samples with a mean concentration of 116 mg/l. Some of

Table III Molar ratios of protease to albumin in normal serum and bronchial lavage fluids using mean concentrations in each group

Protein	Normal serum	Bronchial lavage
$\alpha_1$ Antitrypsin		
Albumin	0.035	0.04
Antichymotrypsin		
Albumin	0.008	0.01
$\alpha_2$ Macroglobulin		
Albumin	0.044	0.02

concentration in plasma was below the threshold of detectability with the technique utilized

*Homogeneity of  $\alpha_1$ -antitrypsin  
 $\alpha_1$ -antichymotrypsin and the bronchial  
inhibitor in bronchial lavage fluids*

**A** Crossed immunoelectrophoresis of a concentrated pool of bronchial lavage fluids and concentrated individual specimens with antiserum against  $\alpha_1$ -antitrypsin showed one precipitate peak (Fig 1). This  $\alpha_1$ -antitrypsin component showed a slightly retarded electrophoretic mobility compared with native  $\alpha_1$ -antitrypsin. The addition of different amounts of granulocyte elastase to the lavage fluids before immunoelectrophoresis did not change this pattern.

**B**

Corresponding analyses with antiserum against  $\alpha_1$ -antichymotrypsin of the same bronchial lavage fluids showed one  $\alpha_1$ -antichymo-



Precipitate patterns obtained on crossed immunoelectrophoresis of human serum (A) and bronchial lavage fluid (B) using an antiserum against  $\alpha_1$ -antitrypsin. Screen electrophoresis of human serum below for reference.

measurable quantities of one or more protease inhibitors.  $\alpha_1$ -Antitrypsin was detectable in 43 of the 51 samples (84%) with a mean value of 5.5 mg/l.  $\alpha_1$ -Antichymotrypsin was found in 30 specimens of the 51 analysed. The mean value was 2.6 mg/l. The high molecular weight plasma protease inhibitor  $\alpha_2$ -macroglobulin was present in 21 of the 51 subjects (41%) with a mean value of 9.5 mg/l. The lower limits of measurability of these plasma protease inhibitors was about 1 mg/l. The bronchial inhibitor was detected in 39 of 51 lavage fluids analysed (76%). The mean value of these 39 lavage fluids was 15.0 mg/l (lower limit about 1 mg/l).

The ratios of inhibitor/albumin were calculated using the mean values (Table III). The values for bronchial lavage fluids were compared with corresponding quotients for human serum. The bronchial inhibitor

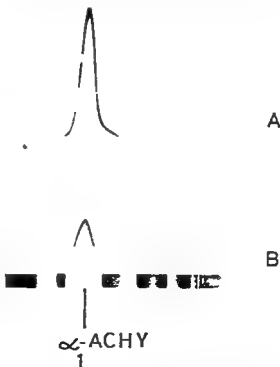


Fig 2 Precipitate patterns obtained on crossed immunoelectrophoresis of human serum (A) and bronchial lavage fluid (B) using an antiserum against  $\alpha_1$ -antichymotrypsin. Screen electrophoresis of human serum below for reference.



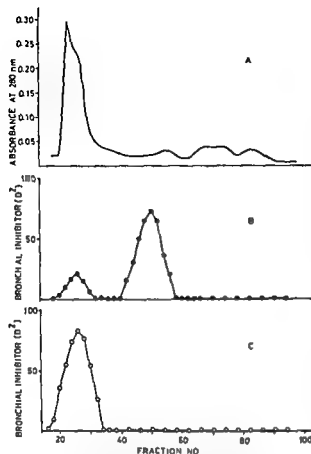


Fig 3 Gel filtration of concentrated bronchial lavage fluid on Sephadex G 75 (A) with the bronchial protease inhibitor localized (B) with the single radial immunodiffusion method of Mancini using antiserum against the bronchial inhibitor. In (C) the bronchial inhibitor is localized in concentrated bronchial lavage fluid which had been incubated with granulocyte elastase in excess prior to the gel filtration

trypsin component (Fig 2). This had the electrophoretic mobility of native inhibitor. The addition of different amounts of chymotrypsin-like cationic proteins to the lavage fluids before analyses did not change this pattern.

On gel filtration of bronchial lavage fluids on Sephadex G-150 antitrypsin eluted in a volume corresponding to a molecular size of about 50 000. The corresponding value for  $\alpha_1$ -antichymotrypsin was 70 000. The addition of different amounts of granulocyte elastase or chymotrypsin-like cationic proteins respectively before analysis did not change these elution patterns.

Unfortunately, the pool of bronchial lavage

fluids available was too small to allow detailed studies of the homogeneity of  $\alpha_1$ -antitrypsin.

On gel filtration of pooled, concentrated bronchial lavage fluid on a calibrated Sephadex G-75 column 85% of the bronchial inhibitor eluted at a volume corresponding to the molecular weight of free inhibitor (10 500) (Fig 3). The remaining 15% eluted at a volume corresponding to a molecular weight of about 40–50 000. After addition of granulocyte elastase to the lavage fluid all of the inhibitor eluted at a volume corresponding to the complexed molecular weight of 40–50 000.

## DISCUSSION

With the exception of the immunoglobulin IgA, no evidence for a local product of serum proteins in the lung has been presented (Asofsky & Thorbecke, 1961; Martinez Tello et al., 1968). In the case of IgA subunit plasma cells have been shown to contain amounts of this protein by

technique (Martinez-Tello et al., 1968). From results of quantitative immunoelectrophoretic studies Ryley & Brogan (1973) recently suggested that a selective concentration of  $\alpha_1$ -antichymotrypsin occurred in the sputum of patients with chronic bronchitis. This observation is of interest in view of the recent finding (Ohlsson & Åkesson 1976) that  $\alpha_1$ -antichymotrypsin is a strong inhibitor of chymotrypsin-like cationic proteins in granulocytes. Furthermore, it is a rapidly responding acute phase reactant, increasing to high concentrations during the granulocyte phase of inflammatory reactions (Aronsen et al., 1972).

Our study utilized bronchial lavage fluid from lungs without signs of clinical bronchitis. No comparison was made of the inhibitor concentration of the lavage fluids and of serum specimens. Instead, a comparison of the ratios of albumin to the three plasma protease inhibitors,  $\alpha_1$ -antitrypsin,  $\alpha_1$ -antichymotrypsin, and  $\alpha_1$ -antitrypsin, was made.

trypsin and  $\alpha_2$  macroglobulin in the lavage fluids and in normal serum was utilized to demonstrate no selective concentration of these proteins (Table III)

Because purulent bronchial secretions usually contain free proteolytic activity (Ohlsson & Tegner, 1975) the protease inhibitors are saturated. Thus the molecular properties of inhibitors in such secretions may be uncertain. The inhibitors may be bound to protease or partially degraded. Those possibilities would introduce error in immunochemical quantitation using free native proteins as standards. The proposed selective concentration of  $\alpha_1$  antichymotrypsin in the bronchial secretions of bronchitis patients (Riley & Brogan, 1973) will require more detailed studies to be confirmed.

The plasma protease inhibitors accounted for only about 10% of the total molar concentration of granulocyte protease inhibitors studied (Table II). The remaining 90% was due to the low molecular weight bronchial inhibitor. Gel filtration studies showed that about 85% of the bronchial inhibitor was present as free native inhibitor with the ability to bind granulocyte elastase. Thus the quantitative measurements of its concentration in the bronchial lavage fluids are valid. The bronchial inhibitor is not measurable in serum by immunodiffusion techniques utilizing the specific antiserum recently produced (Ohlsson & Tegner, 1976). Furthermore, its suggested cross reactivity with the inter  $\alpha$  trypsin inhibitor (Hachstrasser et al., 1973) has not been verified (Ohlsson & Tegner, 1976). A local production of the inhibitor seems likely. A recent study (Tegner & Ohlsson, 1977) using an indirect immunoperoxidase technique localized large amounts of the inhibitor in the ciliated columnar epithelium of trachea and in the seromucous glands. This certainly strengthens the hypothesis of a local production of the inhibitor in the respiratory tract.

A few recent studies (Cohen, 1975; Johnson & Travis, 1976) indicate that  $\alpha_1$  antitrypsin can only bind a protease once. Thus  $\alpha_1$  anti-

trypsin molecules released from their complexes with proteases after transfer of the protease to  $\alpha_2$ -macroglobulin, for example, would be expected to demonstrate no further inhibitory capability. This  $\alpha_1$  antitrypsin component shows a slightly retarded electrophoretic mobility when compared with native  $\alpha_1$  antitrypsin, an altered mobility similar to the mobilities of  $\alpha_1$  antitrypsin in the samples in this study (Fig. 1). The gel filtrations of the lavage fluids after the addition of elastase and chymotrypsin like proteins respectively proved the non reactivity of the major portions of  $\alpha_1$ -antitrypsin and  $\alpha_1$ -antichymotrypsin present. Such non reactive  $\alpha_1$ -antitrypsin was found in large amounts in purulent bronchial secretions in an earlier study (Ohlsson & Tegner, 1975). Furthermore, elastase and collagenase in complex with  $\alpha_1$  antitrypsin and as free protease was present in the purulent secretions. In the present study the major part of the bronchial inhibitor was free. One explanation of the non reactivity of  $\alpha_1$ -antitrypsin and  $\alpha_1$  antichymotrypsin might be that they represent the inhibitor partners of previous complexes with granulocyte proteases normally released from granulocytes on the mucous membranes of the respiratory tract. The proteases may have been transferred to the bronchial inhibitor or  $\alpha_2$  macroglobulin which is eliminated rapidly. This hypothesis would be compatible with the observation that the bronchial inhibitor is partially complexed in the lavage fluids. Further studies of the interactions between the bronchial inhibitor and the plasma protease inhibitors and granulocyte proteases will be necessary to clarify the present findings.

According to preliminary results granulocyte elastase inhibits the mucociliary activity of tracheobronchial epithelium and causes a digestion of the epithelium with time (Tegner, 1977). This study suggests that a low molecular weight protease inhibitor probably produced locally may be the major component of defence mechanism against proteolytic damage of the respiratory mucosa by granulocyte proteases.

## ZUSAMMENFASSUNG

Die bedeutendsten Inhibitoren von Granulozyten Proteasen aus dem Plasma ( $\alpha_1$ -Antitrypsin,  $\alpha_1$ -Antichymotrypsin)

... des Gehaltes von einem niedermolekularen, sauren stabilen Proteaseinhibitor statt. Die Wirkung des Inhibitors richtet sich gegen elastolytische und chymotrypsin-ähnliche Enzyme der Granulozyten. Dieser Inhibitor machte etwa 90% der gesamten proteolytischen Aktivität aus.

... spalt ... sachlich aus  $\alpha_1$ -Antitrypsin und  $\alpha_1$ -Antichymotrypsin. Etwa 8 ... in freie ...

... des immunreaktiven Inhibitors wiesen ein Molekulargewicht auf, das eine Kopplung zu Enzymen vermuten lässt. Der grösste Anteil des  $\alpha_1$ -Antitrypsins und  $\alpha_1$ -Antichymotrypsins wiesen Elektrophoresismuster und Molekulargewichte auf, die den nativen Proteinen ähneln, jedoch liess sich keine Reaktion mit Enzymen nachweisen.

## REFERENCES

- Anzai, T., Ibayashi, J., Carpenter, C. M. & Hyde, L. 1963 Beta 2A globulin as a molecular constituent of insoluble bronchial mucus gel. *Am Rev Resp Dis* 88, 503.
- Aronsen, A. F., Ekelund, G., Kindmark, C. O. & Laurell, C. B. 1972 Sequential changes of plasma proteins after surgical trauma. *Scand J Clin Lab Invest* 29, suppl 124, 127.
- Asofsky, R. & Thorbecke, G. J. 1961 Sites of formation of immune globulins and of a component of C'3. Part II. Production of immunoelectrophoretically identified serum proteins by human and monkey tissue in vitro. *J Exp Med* 114, 471.
- Bisette, G., Havez, R. & Cuvelier, R. 1963 Les glycoprotéides des sécrétions bronchiques. *Exposés Ann Biochim Med* 24, 85.
- Cohen, A. H. 1975 The interaction of  $\alpha_1$ -antitrypsin with chymotrypsin, trypsin and elastase. *Biochem Biophys Acta* 391, 191.
- Dennis, E. G., Hornbrock, M. M. & Ishizaka, K. 1964 Serum proteins in sputum of patients with asthma. *J Allergy* 35, 464.
- Ganrot, P. O. 1972 Crossed immunoelectrophoresis. *Scand J Clin Lab Invest* 36, 437.
- Holm, G. 1975 Inhibitors aus menschlichem Bronchialsekret. *Hoppe-Seyler's Z Physiol Chem* 353, 221.
- Hochstrasser, K., Reichert, R. & Heimburger, H. 1973 Antigenic relationship between the human bronchial mucus inhibitor and plasma inter- $\alpha$ -trypsin inhibitor. *Hoppe-Seyler's Z Physiol Chem* 354, 587.
- Ibayashi, J., Anzai, T., Hood, J. F., Hyde, L. & Carpenter, C. M. 1963 Immunochemical analysis of bronchogenic carcinomatous sputum with special reference to secretin specific proteins. *Dis Chest* 514.
- Johnson, D. A. & Travis, J. 1976 Human  $\alpha_1$ -antitrypsin inhibitor mechanism of action: evidence for activation by limited proteolysis. *Biochem Biophys Res Commun* 72, 33.
- Laurell, C. B. 1972 Electrophoretic assay. *Scand J Clin Lab Invest* 29, suppl 124, 21.
- Laurell, C. B. & Jeppsson, J.-O. 1975 Protease inhibitors in plasma. *The Plasma Proteins* (ed F. H. Pate), vol 1, 2nd edn pp 229-264. Academic Press, New York.
- Lebas, J., Laine, A. & Hayem, A. 1975 Les enzymes du mucus bronchique. *Lille Medical* 21, 136.
- Mancini, G., Carbonara, A. O. & Heremans, J. F. 1961 Immunochemical quantitation of antigens by rapid radial immunodiffusion. *Immunochimistry* 2, 231.
- Mason, P. L., Heremans, J. F. & Pignatelli, B. 1970 Studies on the proteins of human bronchial secretions. *Biochim Biophys Acta* 111, 466.
- Ohlsson, K. 1975 Granulocyte collagenase and its interactions with  $\alpha_1$ -antitrypsin and  $\alpha_1$ -antichymotrypsin. In *Proteases and Biological Control* (ed E. Reich, D. M. Rifkin & E. Shaw) pp 591-602. Cold Spring Harbor Laboratory.
- Ohlsson, K. & Olsson, I. 1973 The neutral proteases of human granulocytes. Isolation and partial characterization of two granulocyte collagenases. *Europ J Biochem* 36, 473.
- 1974a The neutral proteases of human granulocytes. Isolation and partial characterization of granulocyte elastases. *Europ J Biochem* 42, 319.
- 1974b Neutral proteases of human granulocytes. I. Interaction between human granulocyte elastase and plasma protease inhibitors. *Scand J Clin Lab Invest* 34, 349.
- Ohlsson, K. & Tegner, H. 1975 Granulocyte collagenase and plasma protease inhibitors in sputum. *Scand J Clin Lab Invest* 35, 221.
- 1976 Inhibition of elastase from granulocytes by low molecular weight bronchial protease inhibitors. *Scand J Clin Lab Invest* 36, 437.
- Ohlsson, K., Tegner, H. & Åkesson, U. 1974 Isolation and partial characterization of a low molecular weight acid stable protease inhibitor from human bronchial secretion. *Hoppe-Seyler's Z Physiol Chem* 354, 587.
- Ohlsson, K. & Åkesson, U. 1976  $\alpha_1$ -Antichymotrypsin interaction with cationic proteins from granulocytes. *Clin Chim Acta* 72, 285.
- Olsson, I. & Venge, P. 1974 Cationic proteins of human granulocytes. II. Separation of the cationic proteins from the granules of leukemic myeloid cells. *Blood* 44, 724.
- Rothschild, M. A., Oratz, M. & Schreiber, S. S. 1971 Albumin synthesis. *N Engl J Med* 284, 724.
- Ryley, H. C. & Brogan, T. D. 1973 Quantitative immunoelectrophoretic analysis of the plasma proteins of sputum from patients with chronic bronchitis. *J Clin Pathol* 26, 832.

Venge P, Olsson I & Odeberg H 1975 Cationic proteins of human granulocytes. V. Interaction with plasma protease inhibitors. *Scand J Clin Lab Invest* 35: 737.

358-425

Tegner H, Ohlsson K & Olsson I 1977 The interactions between a low molecular weight protease inhibitor of bronchial mucus and chymotrypsin-like cationic proteins of granulocytes. *Hoppe Seyler's Z Phys Chem* 358: 431.

H. Tegner M.D.  
Dept of Otorhinolaryngology  
Malmö General Hospital  
S-214 01 Malmö  
Sweden

## DESQUAMATION ON TASTE BUDS

M Ciges, M Gonzalez and A Ceballos

*From the Department of Otorhinolaryngology, Faculty of Medicine  
University of Granada Granada Spain*

(Received February 23 1977)

**Abstract** Taste bud surface cells suffer from a desquamation process that modifies the pores shape and size and the amount of amorphous substance in the pits region. We have studied this phenomenon—which probably plays an important functional role—by light electron and scanning microscopy.

The taste bud pores and the region of the pit located below, probably play an important role in the first events of the preneural phase of taste. Henkin (1969) believes that there actually exists an open and close mechanism in the pore, regulated by biochemical factors.

Several of us (Ciges et al., 1976) studied the pore region at rest and after stimulation in order to detect morphological changes by light and electron microscopy. We used rabbits, one group was killed in the fasting state and another immediately after stimulation with the taste qualities or normal feeding.

We were able to detect that the pores in the stimulated animals were more frequently open and contained less amorphous substance than did the control group. However, the results were not sufficiently clear to allow any definitive conclusion to be drawn. In fact, opened pores were evident also in the control group.

The tongue has a very unstable mucous membrane that suffers a constant desquamation process and it is subjected to continuous stress by mastication and the various substances with which it comes into contact: alcohol, tobacco, etc. The most changeable

mechanical and therm stimulus are put constantly in contact with the tongue surface.

According to Ceballos & Ciges (1974), desquamation is a very conspicuous process in the tongue surface. It is an intrinsic phenomenon that is obviously modified by extrinsic factors. In fact, they could realize that the desquamation is more evident in omnivorous feeding and various habits in infants.

The morphology and appearance of the tongue's surface depend greatly on this process. The abnormality of the tongue's surface that is most obvious in various pathological conditions is mostly a desquamation, which has not been adequately studied. Probably there exists a biological regulating mechanism to account for it.

We wondered if the physiological stimulation process could affect the surface of the buds in the same way as in the rest of the tongue. Consequently we studied the process by using light, electron and scanning microscopy in a combined approach in which the latter was particularly useful.

## MATERIAL AND METHOD

Fourteen adult rabbits were used: 6 for light microscopy study, 8 for electron microscopy and 2 for the scanning study.

In each case the animals were killed by



Fig 1 Taste buds occupying the whole thickness of an orthokeratinized epithelium (semithin section, toluidine blue staining  $\times 45$ )

Fig 2 Taste papillae lateral surfaces with taste buds and

desquamated debris in its lumen (semithin section, toluidine blue staining  $\times 90$ )

Fig 3 The pore region with nuclei which are very obvious keratinized cells in its periphery (arrows) (EM  $\times 18000$ )

capitation. The tongues were removed easily in order to obtain the foliate papillae. Six of our animals had formed the control group of a previous experiment.

For the optical examination we used hematoxylin-eosin staining, Mallory's method and semithin sections. These sections were prepared from the entire surface of the block for electron microscopy and cut in 0.5 or 1  $\mu\text{m}$  sections. 1% toluidine blue in borax was used as stain.

The specimens for electron microscopy were cut in a prismatic manner keeping a constant orientation reference. 2.5% glutaraldehyde and 1% osmium tetroxide were used for fixation with a common buffer system (phosphate solution according to Millonig, pH 7.4). Acetone was used for dehydration

and the specimens were embedded by Spurr's method.

Ultrathin sections and thicker (0.5 or 1  $\mu\text{m}$ ) sections were cut on an JKB Ultratome with glass knives at 45°. They were examined in a JEM 100B electron microscope.

For scanning electron microscopy, 1% osmium tetroxide was used for fixation and acetone for dehydration purposes. Obtained the specimen under stereoscopic microscope control was evaporated in high vacuum and covered with a thin gold-palladium film and studied by SEM JEOL JSM U.

## RESULTS

In our material studied by light microscopy we can see that the taste buds occupy the whole thickness of the epithelium. This epithelium



Fig. 4. Taste papilla's surface in which we can see desquamated cells. Some of them are in the periphery of the pore (SEM,  $\times 2000$ )



Fig. 5. Taste papilla's surface without desquamated cells. Pores of varying size can be seen, together with microvilli (SEM,  $\times 6300$ )

g. 1) is orthokeratinized, and thinner than one on the outer surface. The surface keratinized layers are very evident, though thin. We could see some desquamated cellular elements in the lumen of the circular groove (Figs. 1 and 2). The keratinized layer is interrupted in the taste pores (Figs. 1 and 2).

In the deepest part of the groove, where the serous glands of von Ebner are usually open (Fig. 2), the epithelium is thicker and the keratinization process less evident. Sometimes a para-keratinized covering epithelium was observed, but never a non-keratinized one.

With the electronmicroscope we could observe a typical stratification in the layers of the epithelium surrounding the taste buds.

In the granular layer the keratohyalin granules were evident and desmosomes were conspicuous.

The keratinized layer (Fig. 3) was very integrated only by two or three cell strata—in some specimens, only one devoted nucleus, organelles or tonofilament. There was a very clear-cut change at the junction of these layers.

In the micrograph from the surface studied by scanning electronmicroscopy we can see different features of great interest.

The surface view with scanning electronmicroscopy allows us to study both the pores and the desquamation process. The shape and size of the pores are very changeable and some of them are covered by desquamated cells. In Fig. 4, we have a view of a papilla, in which we see the covering epithelium is a keratinized layer. The squamulae have an irregular shape and some of them (see the arrows in the micrograph) form the periphery of the pore. These squamulae correspond to the keratinized layer.



Taste pore without desquamated cells. It is open void of amorphous substance. Some microvilli are visible. (SEM  $\times 10000$ )

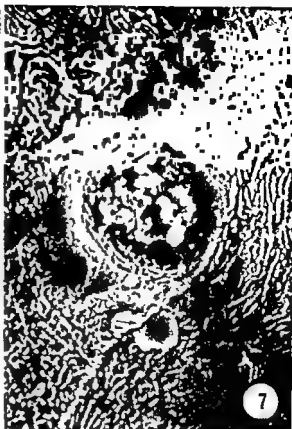


Fig 7 Amorphous substance outside of a pore (arrows). (SEM  $\times 16000$ )

ously by electron microscopy. Some papillae are occupied by an amorphous substance and others devoid of it. In Fig 5 we see another aspect of the papillar surface in which there is less desquamation. However, we also observe differences between the taste pores: in some areas the desquamation process does not exist and in these circumstances the pores are usually open and devoid of amorphous substance (Fig 6). Sometimes we saw amorphous substance inside the pore, an aspect that we can see in Fig 7.

## DISCUSSION

We have been able to observe that desquamation phenomena are very evident in the

papillae that have taste buds. This epithelium is quite similar to that in the remainder of the ortho-keratinized epithelium in the oral cavity, though Squier et al (1976) say that the lateral walls of the papillae are non keratinized and taste buds are present.

If we consider the embryological facts—and also according to Farbman (1971)—there is little doubt that the component cells of the taste buds are derived from the epithelium. In fact, the epithelium is previous to the buds and the nerves induce it to change in some places in order to form the buds. Consequently we must consider that the background of the taste buds is an ortho-keratinized epithelium similar to the one on the outer surface of the papilla.

Looking at our light micrographs we can see how the epithelium with taste buds has a nor-



mal stratification process: basal layer, prickly cells, granular, keratinized layers. However, the taste buds prevent it as foreign bodies.

The final event in taste bud development is the formation of the apical pore, which provides direct communication between taste bud cells and the source of their potential stimuli (Farbman 1971). For Farbman, no conclusive evidence elucidating the mechanism by which the pore is opened and maintained is yet available. For us the mechanism is clear: when the transformation of epithelial cells into sensory cells begins, the keratinization process does not exist and the buds prevent the normal stratification process. Thus, on the surface of every bud, a gap exists in the continuity of the epithelium, i.e. the pore.

The desquamation that we have studied on the surface of the taste buds allows us to understand the variability in the size and shape of the pore. We have noticed that the surface of the taste epithelium suffers the same desquamation process as is seen in any epithelium. Desquamation cells even form the periphery of the pore and when these cells are lost, the diameter of the pores becomes enlarged and the microvilli of the sensory cells are freely exposed to the outer ambient. This is seen not only in our scanning micrographs but also in the electron micrographs.

According to Squier et al. (1975-76) a turnover in the oral epithelia exists, on this turnover depends the stratification and evolution of the epithelia, but this phenomenon is not the same for the whole: no equality of phase exists; on the contrary the process is always in a different phase. So we could see a very distinct aspect of the taste surface and a very different morphology of the pore according to the phase of the desquamation process.

The evolution from the basal layer to the surface is a constant phenomenon. When the surface cells on the periphery of the bud are lost, the pore becomes wider and it is consequently easy for the amorphous substance to pass out of the pore (Fig. 7) because it loses its container.

We have come to the conclusion that the desquamation process alters the morphology of the pore and the amount of its amorphous substance content.

It is probable that the functional states differ according to the degree of desquamation. The pores seen in Figs. 6 and 7 have microvilli in a more accessible position to be stimulated than those shown in the other micrographs, covered by desquamated cells or occupied by an important amount of amorphous substances.

Beidler (1971) suggested a stimulation model based on a mechano-chemical mechanism. According to this hypothetical model, the microvilli of the sensory cells have cylindrical pores filled with minimally cross-linked protein molecules that respond to chemical changes in the environment with a variation of its molecular volume. If we consider this stimulation mechanism, contact of the tastants with the surface of the microvilli would be necessary. In that case a pore without desquamated cells and amorphous substance would be in the best situations to be stimulated.

We could not demonstrate clearly in a previous investigation an open mechanism of the pore with the stimulation. Perhaps this mechanism does not exist or else it is only active at a molecular level as Beidler suggests, or perhaps our stimulation method was not appropriate, as it consisted in pouring the test substance into the mouth of the animal. With this method there is no real possibility of measuring the effectiveness of the stimulation.

Although it was not possible to demonstrate a rapid open mechanism of the pore in direct relation to the stimulus, we have been able to demonstrate a slow regulation of the pore size by the desquamation process. This process must be regulated by different local and general abstract factors. Guth (1971) observed the extrusion of debris through the taste pore and desquamation of degenerating taste buds from the surface epithelium in denervation.

the buds Ceballos & Ciges (1974) saw the same phenomenon in normal tongue from different peoples and others. So we opted for a trophic and direct effect of the nerve in the way of the desquamation process. On the other hand Vicente & Ciges (1975) studied the taste sense in different clinical pathological tongue conditions and they observed that in the cases with an increase of epithelial debris on the surface of the tongue there was a relative hypogoustia.

## ACKNOWLEDGEMENT

We want to express our gratitude to Prof. H. Engström for the facilities given to obtain the scanning electron micrograph in his laboratory at Uppsala University.

## ZUSAMMENFASSUNG

Oberflächenzellen der Geschmacksknospen erleiden Desquamation. Die Form und Größe der Pore sowie die Menge an formloser Substanz verändert. Wir haben diesen Vorgang—der wahrscheinlich eine wichtige funktionelle Rolle spielt—mit der Rasterelektronenmikroskopie sowie mittels der Licht- und Elektronenmikroskopie studiert.

## REFERENCES

- Engström L. M. 1971 Taste receptor stimulation with salts and acids. In *Handbook of sensory Physiology* Vol. IV Taste. Springer Verlag Berlin Heidelberg New York.
- Ceballos A. & Ciges M. 1974 Estudio del ciclo vital de los botones gustativos en la especie humana. *Ann Esp Odontol* 33: 81.
- 1974 Morfología de las papilas gustativas y descamación del epitelio lingual. *Ann Esp Odontol* 33: 437.
- Ciges M., Díaz Flores L., González M. & Rama J. 1976 Ultrastructural study of taste buds at rest and after stimulation and comparative study between type III cell and Merkel cells. *Acta Otolaryngol* (Stockh) 81: 209.
- Farbman A. I. 1971 Development of the taste bud. In *Handbook of Sensory Physiology* Vol. IV Taste. Springer Verlag Berlin Heidelberg New York.
- Guth L. 1971 Degeneration and regeneration of taste buds. In *Handbook of Sensory Physiology* Vol. IV Taste. Springer Verlag Berlin Heidelberg New York.
- Henkin R. I. 1969 The molecular basis of taste and its disorders. *Ann Int Med* 71: 791.
- Squier C. A., Johnson N. W. & Hackermann M. 1975 Structure and function of normal human oral mucosa. In *Oral Mucosa in Health and Disease* (ed. A. E. Dolby). Blackwell Scientific Publ. Oxford London Edinburgh Melbourne.
- Squier C. A., Johnson N. W. & Hopps R. M. 1976 *Human Oral Mucosa*. Blackwell Scientific Publ. Oxford London Edinburgh Melbourne.
- Vicente M. & Ciges M. 1975 Patología lingual y sentido del gusto. *Acta Otorrinol Esp* 26: 136.
- M. Ciges M.D.  
Dept of Otorhinolaryngology  
Faculty of Medicine  
Granada  
Spain

A SIMPLE DEVICE MEASURING DIFFERENCES IN LEVEL  
IN THE OESOPHAGUS

P Ask and L Tibbling

*From the Departments of Medical Engineering and Otolaryngology, University Hospital Linköping, Sweden*

(Received June 27 1977)

**Abstract** A device for measuring the difference in level between the pressure transducer and a point of measurement is described. It can be used in oesophageal manometry with waterfilled catheters to measure and compensate for superimposed hydrostatic pressure. The practical application of the method is illustrated.

Accurate measurement of oesophageal pressure using waterfilled catheters has hitherto been possible only with the patient in supine position (Dodds et al., 1976a, Dodds, 1976b). This is because differences in level between the transducer and the point of measurement in the oesophagus result in a hydrostatic pressure added to the oesophageal pressure. With the patient in the supine position these variations have been considered negligible.

To enable measurement to be made of the difference in level between the pressure transducer and a point of measurement in the oesophagus, a device with a passive catheter has been developed.

## METHOD AND RESULT

The principle of the passive catheter is shown in Fig. 1. The device consists of a U tube, one end of which is filled with water, the other being air-filled and freely communicating with the atmosphere. The shanks of the passive catheter are polyethylene catheters (PF 100, Portex, Great Britain, inner diameter = 0.9 mm, outer diameter = 1.5 mm, length = 1.5 m)

connected to a U shaped tube of stainless steel (inner diameter = 0.3 mm, outer diameter = 1.0 mm, total length = 30 mm). When measuring oesophageal pressure, the passive catheter is applied to a conventional measurement catheter. The transducers of the passive and measurement catheters are kept on the same level. The hydrostatic pressure,  $p$ , measured with the passive catheter, is directly proportional to the difference in level  $\Delta h$  between the air-to-liquid surface and the transducer in accordance with the equation

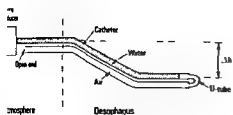
$$p = \Delta h \rho g$$

where  $\rho$  is the density of the catheter fluid and  $g$  the gravitation constant.

Displacement of the air-to-liquid interface of the passive catheters owing to variations in temperature and catheter compliance give an error of measurement but with the catheter used this error is negligible. The error due to temperature variations is approximately 0.6 mmHg/°C, and that due to compliance approximately 0.1 mmHg/m difference in vertical level.

To measure the difference in level in the oesophagus the passive catheter was added to a conventional bundle of three PP 160 catheters with inner diameter 1.0 mm, outer dia-

This work was supported by grants from the Swedish Board for Applied Research No. 75-3447 and the Swedish Medical Research Council No. 178-4'60.



1 The principle of the passive catheter

ter 1.5 m and with side holes. The catheters were connected to transducers (SE Lab tones Feltham, Great Britain) and a three-channel low compliance perfusion pump giving a flow rate of 0.5 ml/min (Ask et al., 1976).

Fig. 2 shows examples of intra-oesophageal pressures in vertical level measured with the passive catheter in a recumbent patient. The oesophagus level in relation to the level of the lower oesophageal sphincter (LES) varies in the range 0 to 25 mm. Measurements of oesophageal pressures made with stepwise pull through from the stomach to the distal part of the oesophagus in a sitting patient are given in Fig. 3. The recordings show pressures measured with the perfused catheter and the same pressures after compensation for differences in level measured with the aid of the passive catheter.

## CONCLUSION

The device with a passive catheter makes it possible to measure differences in vertical level between the pressure transducer and the point of measurement in the oesophagus. By subtraction of the pressure measured with the passive catheter from the pressure

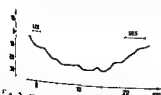


Fig. 2 The difference in vertical level (mm) in the oesophagus expressed as a function of the distance (cm) from the lower oesophageal sphincter (LES) to a point of measurement in a recumbent patient. UES, upper oesophageal sphincter.

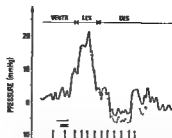


Fig. 3 The intra-oesophageal pressure in a sitting patient. Each pull through step (1 cm) from the ventricle (VENTR) through the lower oesophageal sphincter (LES) to the oesophagus (OES) is indicated by an arrow. The graphs show the measured intra-oesophageal pressure (—) and the same pressure after compensation for differences in level measured with the passive catheter (---).

with the measurement catheter the intra-oesophageal pressure in relation to the atmospheric pressure is obtained. By this means it is possible to measure the pressure in all body positions and also to measure the divergence of the oesophagus from the direction of the body axis.

## ZUSAMMENFASSUNG

Eine Vorrichtung wird beschrieben, mit der man Niveauunterschiede zwischen einem Druckgeber und einem Meßpunkt feststellen kann. Diese kann bei Oesophagusmanometrie mit wassergefüllten Kathetern verwendet werden, um den überlagerten hydrostatischen Druck zu messen und zu kompensieren, was illustriert wird.

## REFERENCES

- Ask P, Tibbling L & Öberg P Å. Compliance and bandwidth of oesophagus manometry systems. *Digest of the International Conference on Medical and Biological Engineering* (Ottawa 2-6 August 1976) pp 688-689.
- Dodds W J, Stef J J & Hogan W J 1976a Factors determining pressure measurement accuracy by intraluminal esophageal manometry. *Gastroenterology* 70: 117.
- Dodds W J 1976b Instrumentation and methods for intraluminal esophageal manometry. *Arch Intern Med* 136: 515.

L. Tibbling MD  
Dept of Otolaryngology  
University Hospital  
S 58185 Linköping  
Sweden

## ULTRASTRUCTURAL OBSERVATIONS ON THE PAROTITIS AUTOIMMUNICA IN THE NZB/NZW HYBRID MICE

B Carlsoo and Y Östberg

*From the Departments of Histology, Otolaryngology and Pathology  
University of Umeå, Umeå, Sweden*

(Received March 4 1977)

**Abstract** The lesions of the spontaneously occurring sialoadenitis in the parotid gland of New Zealand Black/New Zealand White (NZB/NZW) hybrid mice were studied with the light microscope and the electron microscope. The inflammatory mononuclear cell infiltrates of the glands observed adjacent to glandular vessels and ducts were found to consist of several different cell types. Lymphoid cells (large medium sized and small lymphocytes) were the most common but plasma cells histiocytes and macrophages were also encountered. Further more lymphoid cells were frequently observed inside the basement membrane of acini as well as intercalated ducts. Evidence of cell damage of the epithelium invaded by lymphoid elements included loss of cytoplasmic density vesiculation of the endoplasmic reticulum the appearance of cytoplasmic vacuoles and cellular lysis. All the animals studied displayed moderate to advanced glomerular wire loop lesions in the kidneys. The light and electron microscopic findings indicate that the parotitis in NZB/NZW mice is basically a vasculitis. Duct cell proliferation and epimyoe-epithelial island formation which are common features in Sjogren's syndrome are totally lacking in the material studied.

The autoimmune sialoadenitis (Mikulicz's disease benign lymphoepithelial lesion) in Sjogren's syndrome constitutes part of a systemic disease of considerable rheumatological and immunological interest (cf Shearn 1971 Mason & Chisholm 1975). The basic pathological changes occurring in the salivary glands of Sjogren's syndrome are however poorly understood (Boquist et al 1970 Kitamura et al 1970 Donath & Seifert 1972 Pirsig & Donath 1972). This is partly ex-

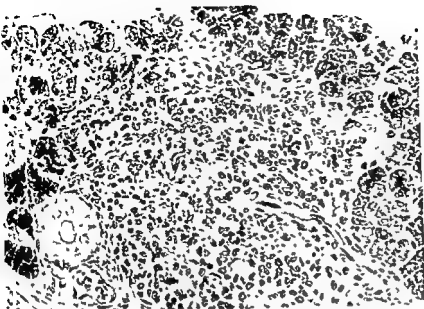
plained by difficulties in making early diagnosis and thus obtaining biopsies from the salivary glands at early stages of the disease. The report of a murine type of Sjogren's syndrome (Kessler 1968 Kessler et al 1971) is therefore of great interest and importance. However, to the best of our knowledge there are no ultrastructural reports on cellular features in the salivary glands of this laboratory animal model of Sjogren's syndrome.

The present report is an electron microscopic study on the parotid gland in the New Zealand Black/New Zealand White (NZB/NZW) hybrid mice with moderate and advanced kidney involvement (Mellors 1960 Talal & Steinberg 1974).

### MATERIALS AND METHODS

NZB/NZW F1 hybrid mice obtained from the Medical Research Council Laboratories Animal Centre, Carshalton Surrey England were employed for the investigation. Twelve animals (6 females age range 6-8 months 6 males age range 8-10 months) showing signs of terminal stages of their renal disease were fasted overnight and sacrificed. The parotid glands and the kidneys were removed.

This work has been supported by grants from the Swedish Medical Research Council (Project No B77 19X-0400) and the Mångberg Fund Umeå.



1 One micron thick Epon section of the parotid gland stained with toluidine blue. A large mononuclear infiltrate is observed in the central portion of the

section. The surrounding acinar and stratified duct cells appear comparatively unaffected. Light micrograph  $\times 560$ .



2 Electron micrograph of an inflammatory cell in the parotid gland. Four small lymphocytes (S) as well as medium sized (M) and a large lymphocyte (L) are depicted in the section  $\times 5300$ .

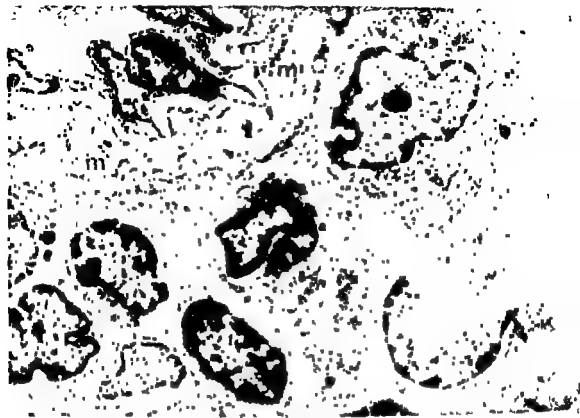


Fig 3 Electron micrograph of lymphoid cells surrounding a small arteriulus. The cells are seen in the adventitia

of the vessel in close proximity to the muscularis (m)  $\times 5100$

diately removed from the animals and processed for the subsequent microscopical studies. All animals used in this investigation showed glomerular wire-loop lesions of varying degree. The light microscopical and ultrastructural features of their autoimmune nephropathy have previously been reported (Emdin & Östberg, 1972).

#### Light microscopy

Specimens from the parotid glands were fixed in 10% formalin and embedded in paraffin wax. Deparaffinized sections were stained with hematoxylin-eosin and the periodic acid-Schiff (PAS) reaction.

#### Electron microscopy

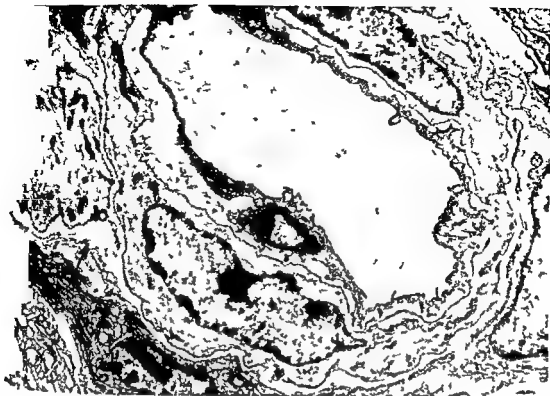
Small pieces of parotid gland and kidney tissue were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 hours at 0–4°C, rinsed in the same buffer,

post-fixed in 1% osmium tetroxide, and embedded in Epon 812. Sections were cut on an LKB Ultratome. Semithin ( $\sim 1 \mu\text{m}$ ) sections were stained with toluidine blue and used for the light microscopic identification of suitable areas for the thin sections. Ultrathin sections were mounted on uncoated copper grids, and double contrasted with uranyl acetate and lead citrate; they were examined in a Philips EM 300 electron microscope.

## RESULTS

#### Light microscopy

The overall lobular architecture of the parotid gland was preserved, and the acinar cells were filled with distinct secretory granules. In some focal areas, however, the gland parenchyma was replaced by lymphoid tissue (Fig 1). The size of the lesions varied from rather conglomerates of lymphoid cells to large areas



Electron micrograph of a small arteriolar traversed by a mononuclear cell aggregate. The external basement

membrane of this vessel is pronounced and thickened (arrows)  $\times 6700$

with diffuse lymphocytic infiltration. The nonnuclear cell aggregates often formed nodular and periaarteriolar nodules. Small islands of degenerated epithelial cells displaying a dark agranular cytoplasm were occasionally found within the lymphocytic aggregates.

#### Electron microscopy

At the ultrastructural level the mononuclear cell aggregates were found to be composed of several different cell types. Small and medium sized lymphocytes were the most conspicuous cells present (Fig. 2). Based on purely morphological grounds, i.e. their size, the lymphoid cells were categorized as small lymphocytes (5–6  $\mu\text{m}$ ), medium sized (6–10  $\mu\text{m}$ ) and large lymphocytes (10–12  $\mu\text{m}$ ). The spherical nuclei of the small lymphocytes displayed a prominent chromatin pattern, nucleoli however were not often seen. In the cytoplasm

free ribosomes were abundant; they were generally dispersed in the fairly electron dense hyaloplasm. In the medium sized lymphocytes the nuclei were often irregular in shape. Their chromatin was less condensed and prominent nucleoli were present (Figs. 2, 3). In the cytoplasm mitochondria were sparse and only short channels of rough surfaced endoplasmic reticulum could be observed. Polynucleosomal clusters were frequently encountered. The large lymphocytes finally displayed a rather electron lucid cytoplasm with a multitude of free ribosomes and sparse serpentine profiles of rough surfaced endoplasmic reticulum. The nuclear heterochromatin was often concentrated to a rather thin peripheral rim (Figs. 2, 3). Frequently the lymphoid cells were difficult to classify and intermediate types were often observed. Mature plasma cells with well developed Golgi regions and stacks of parallel dilated rough surfaced endoplasmic reticulum





Fig 5 Lymphoid cells of medium size (L) invading the epithelium of a secretory acinus. Electron micrograph  $\times 5300$

were also encountered. In some areas they were even abundant. Non-phagocytic connective tissue cells as well as macrophages were also observed within the lymphoid aggregates. Phagosomes containing degenerating cell structures were seen in many of the macrophages. Moreover, within these areas cell debris and necrotic cells abounded.

Encircling many of the small vessels, aggregates of lymphocytes and plasma cells appeared (Fig 3). The cells of these perivascular adventitial nodules were mainly concentrated in close proximity to the muscular media of the arterioli. The external basement membrane of these vessels was frequently extremely well developed and fairly broad (Fig 4).

Lymphoid cells were often interspersed between the epithelial cells of the acini (Figs 5, 6, 7) and also seemed to migrate between the intercalated duct cells (Figs 8, 9). Many of the secretory cells, in close proximity to invading

lymphoid cells, appeared damaged. They displayed degenerative features such as increased cytoplasmic density, dilated or vacuolated endoplasmic reticulum, cytoplasmic vacuolization, and cellular lysis (Figs 5, 6). The agranular dark epithelial cells within lymphoid aggregates observed in the microscope, could at the ultrastructural level be identified as intercalated duct cells (10).

## DISCUSSION

The pathological features in the major salivary glands from patients suffering from Sjögren's syndrome are characteristic. The features include marked lymphocytic infiltration, duct hyperplasia and the presence of so-called epithelial islands (Morgan & Castleman, 1971). The cells of the islands consist of epithelial cells, derived from the ducts as well as



Fig. 6. Electron micrograph of a secretory acinus. Two mononuclear cells are observed insinuating between the epithelial cells. The intercellular spaces are markedly widened. One acinar cell displays dilated cisternae of the rough endoplasmic reticulum and a large vacuole is observed in another acinar cell.  $\times 5100$ .

inflammatory cells (Boquist et al., 1970). In the later stages of the disease there is a destruction of the duct epithelium, proliferation of intercalated duct myo-epithelial cells and infiltration, mainly by lymphocytes (Donath & Pfeiffer, 1972). In the final stages, myo-epithelial cells produce a "basement membrane-like material causing extensive hyalin transformation of the islands. The hyalin deposits, supposed to represent an antigen-antibody reaction, diminish after immuno-suppressive therapy (Pirsig & Donath, 1972).

In the NZB/NZW hybrid mice an autoimmune disease occurs spontaneously (Mellors, 1966; Greenspan et al., 1974a). The animals develop glomerular lesions analogous to those observed in systemic lupus erythematosus (SLE) (Emdin & Ostberg, 1972). Furthermore,

these mice exhibit histo-pathological changes in both salivary and lacrimal glands similar to those observed in the glands from patients with Sjogren's syndrome (Kessler, 1968).

The mononuclear infiltrates in the parotid gland of the NZB/NZW mice (Kessler, 1968) observed in the present investigation were found to consist of a rather heterogeneous cell population. However, lymphocytes of varying size constituted a major portion of the invading cells, but histiocytes, macrophages and plasma cells were also frequently encountered. Lymphocytes were also detected inside the acinar basement lamina and thus in close contact with the acinar cells. The latter often exhibited dilated cisternae of the RER, occasionally an extremely electron-lucid cytoplasm and/or pyknotic nuclei. In addition, infiltrating lymphocytes were also observed in-





Fig 8 A lymphocyte invading an intercalated duct. The lymphoid cell (L) is observed inside the basement membrane of the duct (arrows). Electron micrograph  $\times 10000$

side the basement membrane of the intercalated ducts. It therefore seems reasonable to assume that both the secretory cells and those of the intercalated ducts represent target cells for the infiltrating destructive lymphocytes.

The small cell islands of epithelial cells observed within the lymphoid aggregates could, at the ultrastructural level, be recognized as intercalated duct cells. However, no signs of intraductal cell proliferation and/or formation of myoepithelial cell islands were observed in the parotid glands of NZB/NZW mice, nor were any hyalin deposits present in the gland tissue. Thus, from a histopathological point of view the autoimmune parotitis in NZB/NZW mice is very similar to the human sialoadenitis of the labial salivary glands where myoepithelial cell proliferation also is lacking (Shearn, 1971).

By employing immunofluorescence micro-

scopic techniques, Greenspan and co-workers (1974b) have demonstrated that both thymus-derived lymphocytes (T cells) as well as bone marrow lymphocytes (B cells) are present in the mononuclear cell infiltrates in the salivary glands of the NZB/NZW mice. In the ear gland lesions these authors recorded that lymphocytes of the B cell lineage and plasma cells were the most conspicuous, whereas a great proportion of T-cells was found in later stages when the infiltrates were enlarged. Also in the salivary gland lymphocyte infiltrates in patients with Sjögren's syndrome mixed T- and B cell aggregates have been recorded (Chused et al., 1974; Talal et al., 1974). According to Greenspan et al. (1974) the B cells and plasma cells could be involved with antigen recognition and/or early tissue damage. The T-cells on the other hand



Fig 9 Marked lymphoid infiltration of an intercalated duct. The lymphoid cells have penetrated the basement membrane and the damaged epithelial cells display numerous cytoplasmic vacuoles. Electron micrograph  $\times 5100$



Fig. 10. Portion of a small island of epithelial cells present within a mononuclear cell infiltrate. The lymphoid cells are in close contact with the epithelial cells. The latter display a fairly electron-dense cytoplasm, small Golgi

complexes (b) and a sparse endoplasmic reticulum. Desmosomal attachments are observed along the epithelial cell borders (arrows). Note the presence of two duct lumina (D). Electron micrograph  $\times 8300$ .

become subsequently involved through sensitization as a result of autoantigens release by  $\delta$ -cell cytotoxicity or as non-specific recruits in this delayed inflammatory reaction. By applying immunofluorescence microscopic techniques, it is possible to distinguish between T and B lymphocytes. However, at the ultrastructural level the most common cell in the thymic gland, the thymic lymphocyte or T-cell, is indistinguishable in structure from small lymphocytes elsewhere. Also the lymphocytes of medium and large size within the thymus are structurally similar to medium and large lymphocytes in other lymphoid organs (Rhodin, 1974). Due to the difficulties in detecting differences in lymphocyte morphology by transmission electron microscopy, reports that B and T lymphocytes, examined by scanning electron microscopy (SEM), could be

identified by their distinctive surface morphology, have been met with interest. However, Alexander et al. have recently (1976) reported that it is not possible to reliably identify classes of lymphocytes *in vitro* exclusively on the basis of their SEM surface topography. Thus, further refined electron microscopic immunocytochemical studies are required to reveal the difference between surface immunoglobulin-positive and -negative cells within the different sites in the salivary glands of the NZB/NZW mice.

The histopathological changes occurring in the parotid glands of the mouse strain studied should be referred to as a vasculitis. Although differing in features from the parotitis affection in the human Sjogren's syndrome, the similarities between the major salivary gland lesions in the NZB/NZW mice and those

of the labial salivary glands in patients with Sjögren's syndrome (Greenspan et al., 1974c) suggest common factors in pathogenesis and perhaps even in etiology.

## ACKNOWLEDGEMENT

The skilful technical assistance of Miss Siv Domeij is gratefully acknowledged

## ZUSAMMENFASSUNG

Die Läsionen von spontan auftretender Sialadenitis in der Ohrspeicheldrüse von mischerbigen Neuseeland Schwarz/Neuseeland Weissen (NZB/NZW) Mäusen wurden licht- und elektronenmikroskopisch studiert. Es stellte sich heraus, dass die entzündlichen einkernigen Zellen Infiltrate der Drüsen, die man in der Nähe von glandulären Gefässen und Gruppen beobachtet.

Ausserdem wurden lymphoide Zellen oft innerhalb der Basalmembran von Acini und auch innerhalb der dazwischenliegenden Gänge beobachtet. Der nachweis der Zellenzerstörung des von lymphoiden Elementen durchsetzten Epithels umfasst den Verlust von protoplasmischer Dichte, Blasenbildung des endoplasmischen Retikulums, das Vorkommen von cytoplasmischen Vacuolen und zellige Lyse. Alle untersuchten Tiere wiesen massige bis hohe glomeruläre, drahtschleifige Läsionen in den Nieren auf.

Die licht- und elektronenmikroskopischen Befunde indizieren, dass die Parotis bei NZB/NZW Mäusen in erster Linie eine Vasculitis ist. Gang Zellen Wucherung und epimyoepitheliale Inselformation gewöhnliche Züge beim Sjögren Syndrom sind im untersuchten Material überhaupt nicht vorhanden.

## REFERENCES

- Alexander, E. Sanders S & Braylan, R. 1976. Purported difference between human T cells. *Immunology* 29, 410.
- Boc, J. 1976. *Immunology* 29, 410.
- Chused, T. M., Hardin, J. A., Frank, M. M. & Green, I. 1974. Identification of cells infiltrating the minor salivary glands in patients with Sjögren's syndrome. *J Immunol* 112, 641.
- Donath, K. & Seifert, G. 1972. Ultrastruktur und Patho-

- genese der myoepithelialen Sialadenitis. *Arch Anat Hist A Path Anat* 356, 315.
- Emdin, S. & Östberg, Y. 1972. Scanning electron microscopy of the glomerular lesions in mice with autoimmune nephropathy. *Acta Pathol Microbiol Scand Sect A*, 80, 694.
- Greenspan, J. S., Gutman, G. A., Talal, M., Weissman, I. L. & Sugar, S. 1974a. Thymus antigen and immunoglobulin positive cells in lymph nodes, thymus and malignant lymphomas of NZB/NZW mice. *Cl Immunol & Immunopathol* 3, 32.
- Greenspan, J. S., Gutman, G. A., Weissman, I. L. & Talal, N. 1974b. Thymus antigen and immunoglobulin positive lymphocytes in tissue infiltrates of NZB/NZW mice. *Cl Immunol & Immunopathol* 3, 32.
- Greenspan, J. S. 1974. Sjögren's syndrome in labial salivary gland biopsies. *Oral Pathol* 37, 217.
- Kessler, H. S. 1968. A laboratory model for Sjögren's syndrome. *Amer J Pathol* 52, 671.
- Kessler, H. S., Cubberly, M. & Manski, W. 1971. Eye changes in autoimmune NZB and NZB×NZW mice. *Arch Ophthalmol* 85, 211.
- Kitamura, T., Kanda, T., Ishikawa, T. & Shimizu, T. 1970. Parotid gland of Sjögren's syndrome. *Arch Otolaryngol (Stockh)* 91, 64.
- Mason, D. K. & Chisholm, D. B. 1975. *Salivary Glands in Health and Disease*. W. B. Saunders Comp. London, Philadelphia, and Toronto.
- Mellors, R. C. 1966. Autoimmune and immunoproliferative diseases of NZB/Bl mice and hybrids. *Int Rev Exp Pathol* 5, 217.
- Morgan, W. S. & Castleman, M. 1953. A clinicopathologic study of Mikulicz's disease. *Am J Path* 29, 471.
- Pirsig, W. & Donath, K. 1972. Zur Ultrastruktur der Parotis beim Sjögren Syndrom vor und nach immunsuppressiver Therapie. *Arch Klin Exp Otolaryngol Kehlkopfheilkd* 201, 309.
- Rhodin, J. A. G. 1974. *Histology: A Text and Atlas*. Oxford University Press, London, Toronto and New York.
- Shearn, M. A. 1971. *Sjögren's Syndrome*. vol II. W. B. Saunders Comp., Philadelphia, London and Toronto.
- Talal, N. & Steinberg, A. D. 1974. The pathogenesis of autoimmunity in New Zealand Black mice. In *Current Topics in Microbiology and Immunology* p. 71. Springer Verlag, Berlin Heidelberg, and New York.
- Talal, N., Sylvester, M. A., Daniels, T. E., Greenspan, J. S. & Williams, R. C. Jr. 1974. T and B lymphocytes in peripheral blood and tissue lesions in Sjögren's syndrome. *J Clin Invest* 53, 180.

B. Carlsoo, M.D.  
Dept of Otolaryngology  
University of Umeå  
S-90187 Umeå  
Sweden

# ORGAN CULTURE STUDIES ON HUMAN SKIN AND CHOLESTEATOMA EPITHELIUM

*Contact with Connective Tissue and Exposure to Vitamin A*

T. Palva, I. Thesleff and L. Saxen

*From the Department of Otolaryngology (T. P.) and from the Third Department of Pathology (I. T. & L. S.) University of Helsinki, Finland*

(Received July 20 1977)

*Abstract* In tissue culture trunk skin from 8-day old chick showed a consistent change from keratinizing to more columnar non keratinizing epithelium under the influence of vitamin A acid using concentrations from 10<sup>-6</sup> to 10<sup>-4</sup> IU/ml. Human adult postauricular and ear canal cholesteatoma membrane and human embryonic retained their keratinizing properties when exposed to vitamin A acid at concentrations up to 150 IU/ml when the changes in the epithelium became obvious. Treatment of cholesteatoma ears with vitamin A ear drops is not expected to effect any change in the keratinizing properties of the epithelium.

present, little is known about the mechanisms controlling the development of a given type of epithelium and of the stimuli triggering its metaplastic changes. Fell & Melnick (1953) noted that embryonic chick skin can be transformed from a keratinizing to a mucus secreting epithelium by exposure to excess vitamin A. Weiss & James (1955) found that epidermal cell suspensions exposed to vitamin A only briefly (15 minutes) grow according to the metaplastic pattern even in normal unenriched media. They postulated that this brief contact with vitamin A was sufficient to switch the cell differentiation into an alternative pathway.

In experiments on embryonic rat skin from the tail and pads and from the trunk, New (1965) found 2-3 mm<sup>2</sup> large specimens to undergo certain changes under the effect of vitamin A. Whereas control specimens developed thick stratified epidermis with abundant keratin, vitamin A treated skin either failed to de-

velop keratin, or if formed it was soon shed together with epidermal cells. However, no mucous metaplasia occurred while some goblet cells were seen. Barnett & Szabo (1973) reported inhibition of keratin formation with development of microvilli in the skin taken from the dorsum of a guinea pig's ear.

Another mode of induction into different types of epithelium was noted in 5-day-old chick embryo tissue cultures by McLoughlin (1961). Various connective tissues specifically affected the differentiation and growth of epidermis. On limb mesenchyme epidermis formed as normal, keratinizing epithelium, on gizzard mesenchyme it changed into mucus secreting instead of keratinizing epithelium, on heart myoblasts it grew as a thin 1 cell layer of endothelium type epithelium where as on heart fibroblasts it keratinized more heavily than in the controls.

Experimentally, vitamin A has also been shown to play an important role in wound healing. It stimulates the migration of epidermal cells and if cortisone has been used to impair wound healing, decreasing the strength of collagen, addition of vitamin A normalizes the system immediately (Ehrlich 1976). Vitamin A is thought to be of importance in glycoprotein synthesis and also to function as a carrier for sugar-phosphate compounds across the cell membranes.

The use of vitamin A has also recently been suggested for the treatment of chronic middle

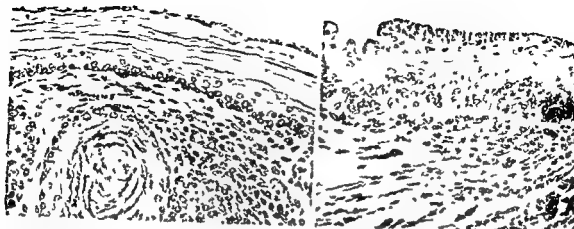


Fig 1 The effect of vitamin A acid on 8-day-old chick embryo trunk skin in 10-day old tissue culture. The control specimen (left) shows strongly keratinizing squamous epithelium, while the specimen exposed to vitamin A acid

in a concentration of 20 IU/ml has lost all keratinized and the epithelium has become more columnar in type.  $\times 300$

ear disease. It has been reported to decrease infection and smell and to push back the cholesteatoma membrane (Duncan, 1976). Rarely, in individual cases, one of us (T. P.) observed disappearance of cholesteatoma epithelial remnants from the oval window when total removal had been judged to be too risky and the epithelial remains had been covered with crushed temporalis muscle tissue piece to await second stage surgery.

In this work we have studied the growth capabilities of human postauricular skin, ear canal skin and cholesteatoma epithelium as well as various embryonic tissues in tissue culture and subjected the specimens to contact with various types of connective tissue as well as to different concentrations of vitamin A acid.

## MATERIAL AND METHODS

The following types of skin cultures were studied:

- 1 trunk skin from 8-day-old chick embryos
- 2 postauricular and canal skin and cholesteatoma membrane from a human adult
- 3 postauricular and canal skin and cholesteatoma

membrane from a human adult cultured on a piece of temporalis muscle or fascia.

4 abdominal skin from a 190 mm CR human embryo. Postauricular and canal skin as well as pieces of temporalis muscle and muscle fascia were removed from ear surgery and placed directly in culture medium. Human embryonic skin was obtained from a case of operative abortion.

The skin was cut in approx.  $2 \times 2$  mm pieces in sterile phosphate buffered saline under a dissecting microscope. A layer of superficial dermis was left under the epidermis. A Trowell type organ culture was used (Saxén, 1966). Here the explants are cultivated on a metal grid at the medium-gas interface in a humidified incubator in an atmosphere of 5%  $\text{CO}_2$  in air at  $37^\circ\text{C}$ . Small pieces of Millipore filter (0.8  $\mu\text{m}$  pore size, 25  $\mu\text{m}$  thickness) were placed on the metal grid and the skin explants were carefully placed on the filters, epidermis upwards. In experiments of the

Altogether 12 explants of chick skin, 56 explants of adult human skin and 20 explants of embryonic human skin were cultivated.

In cultures of chick skin BGJb medium (Biggers et al., 1961; Orion Pharmaceuticals, Finland) supplemented with 10% or 20% horse serum (Flow Laboratories) or with 20% horse serum + 10% chick embryo extract was used. These media were also used for cultivating human adult skin, but equally good growth was obtained using a simpler medium Minimum Essential Medium (MEM) (Orion Pharmaceuticals, Finland) + 10% fetal calf serum (Microbiological Associates Inc., Bethesda, Md.) the lat-

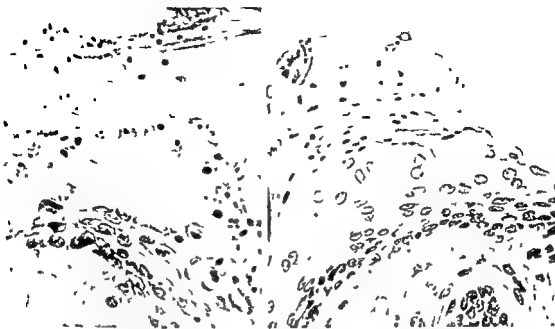


Fig. 2 The effect of vitamin A acid on human adult skin on muscle after 15 days of tissue culture. The control specimen (left) as well as the treated

specimen (40 IU/ml vitamin A acid right) both show keratotic squamous epithelium with clear parakeratotic changes  $\times 300$

um was subsequently used in all experiments with adult as well as embryo skin.

juve skin. Vitamin A acid was first dissolved in absolute ethanol in a concentration of 3 mg/ml. From this solution appropriate amounts were dissolved in serum by simultaneous vigorous shaking, whereafter the serum was added to the medium.

The medium was changed every other day in all experiments. Chick skin was cultivated for 8 days and human skin for 8, 15 and 30 days. The explants were fixed in Zenker's solution and paraffin embedded. The 6  $\mu$ m serial sections were stained with hematoxylin and eosin with PAS or Alcian Blue.

## RESULTS

The experiments showed that all human epidermal tissues studied grew well in the MEM culture medium supplemented with fetal calf serum (FCS) showing good keratinization and forming rounded epidermal pearls. Addition of fascia or muscle as a connective tissue matrix did not alter these properties. Epidermis lined both the fascia and the muscle and in the specimens retained for 3 weeks it had a

tendency to grow around the connective tissue pieces.

Exposure of chick embryo skin to vitamin A acid resulted in a consistent change of the stratified squamous keratinizing epithelium into a more columnar type epithelium with no sign of keratinization (Fig. 1).

Addition of 20% horse serum or 10% embryo extract, separately or together, to the BGJb culture medium did not affect loss of keratinization ability. On the other hand, the change from keratinizing into columnar type epithelium was most marked when 10% embryo extract was used together with 20 IU/ml vitamin A acid.

Staining with PAS gave a faint reddish tinge to the intercellular spaces but no proper secretory cells were seen. All Alcian Blue stainings were negative in regard to acid mucopolysaccharides.

Similar experiments with human epidermis gave negative results. The keratinizing properties of postauricular and canal skin growing by themselves or on muscle bed with added



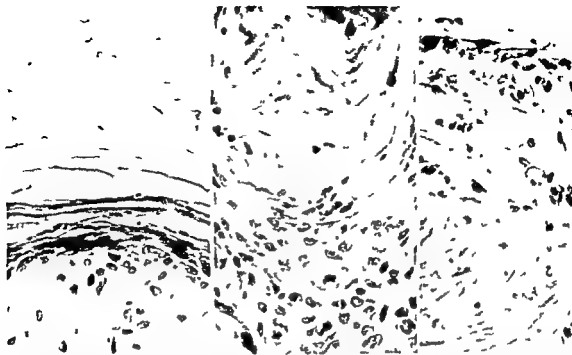


Fig 3 The effect of vitamin A acid on human adult ear canal skin on muscle in 12-day-old tissue culture. The control specimen (left) shows marked keratinization with slight parakeratosis. The specimen treated with vitamin A acid at 100 IU/ml (middle) shows a keratin pearl and some damage to the nuclei in the epithelium. The specimen treated with vitamin A acid at 150 IU/ml (right) shows toxic disintegration of the whole epithelium.

horse serum or embryo extract, or both, were not affected by addition of vitamin A acid in different concentrations (Figs 2 and 3). The toxic effect of vitamin A acid, nevertheless,

became noticeable at 100 IU/ml and even more marked using a concentration of 150 IU/ml. First the epithelial cells in the superficial layers were detached from each other, then

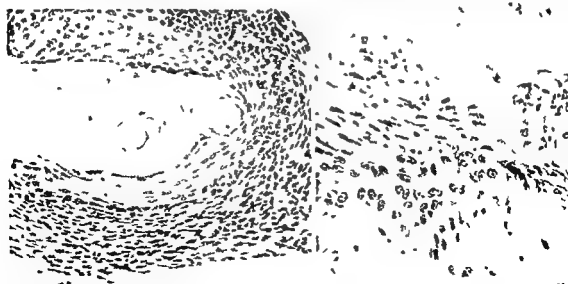


Fig 4 The effect of vitamin A acid on human 190 mm embryo abdominal skin in 15-day-old tissue culture. The control specimen shows abundant keratinization (left).

— vitamin A acid (150 IU/ml)  
--- toxic effect

whole superficial cell layer was separated from normal remaining basal cells and finally whole epithelium disintegrated

The effect of vitamin A acid was then tested skin of a human embryo, epidermis and muscle being taken from the abdominal area grown in MEM +10% FCS. Keratinization was seen to continue unabated at all concentrations and toxic effect of vitamin A acid could be observed using a concentration of 150 IU/ml (Fig. 4)

## DISCUSSION

The effect of vitamin A acid on chick embryo was clear and consistent when the initial experiments were repeated with increasing concentrations of this preparation. However, vitamin A acid did not alter the keratinizing effect of human adult nor even of human embryonic epidermal tissues, the highest concentration used, 150 IU/ml, already exerting a toxic effect upon the epithelium.

The idea of carrying out experiments with the two types of connective tissues, fascia and muscle as a potentially influential matrix for epidermal tissue transformation originally derived from the experiments by McLoughlin, who showed that heart fibroblasts enhanced keratinization and myoblasts changed the epithelium into a thin endothelium type lining. It is precisely with fibroblast rich fascia that squamous epithelium always comes into contact in tympanoplasties, while muscle comes into play only in obliteration procedures. Clinical experience provides ample evidence that fascia is an ideal bed for resurfacing with keratinizing epidermis and this was fully borne out by the tissue culture experiments. Unfortunately, however, neither crushed nor uncrushed muscle affected the keratinizing epithelium in any way. It may be postulated that the tissue culture medium rapidly removes the possible enzymatic factors capable of epidermal transformation in crushed muscle whereas *in vivo* these factors might be retained long enough in a sufficiently high concentration to

provide a booster for transformation of small remnants of squamous epithelium. In this respect, clinical experiments must be continued.

At present, it seems obvious that *in vitro* fascia and muscle do not affect the properties of keratinizing epithelium, while vitamin A acid consistently transforms keratinizing embryonic chick epidermis into a nonkeratinizing type. This effect does not apply to human embryonic or mature keratinizing human epidermis and it is not likely that vitamin A ear drops at a non-toxic concentration could exert any definite effect on cholesteatoma epithelium in chronic ears. Some other factors govern the transformation of epithelium and their discovery would indeed greatly influence treatment of middle ear problems.

## ZUSAMMENFASSUNG

In Gewebeskultur verursachte die A-Vitaminsäure (Konzentration 10–20 IU/ml) eine Transformation des keratinisierenden Plattenepithels zu nichtkeratinisierendem kolumnarem Epithel bei Hautspezimens aus 8 Tage alten Hühnerembryonen. Die postaurikuläre und Ohrenkanalhaut Kolestomatopithel und auch die embryonische Haut waren unverändert gleich unter Wirkung von A-Vitaminsäure bis Konzentration zu 150 IU/ml, wenn schon die toxischen Effekte der Säure sichtbar wurden. Es ist unwahrscheinlich, daß die A-Vitaminsäure einen klinischen Effekt auf die Keratinisierung des Epithels verursachen konnte.

## REFERENCES

- Barnett M L & Szabo G 1973 Effect of vitamin A on epithelial morphogenesis *in vitro*. *Exp Cell Res* 76: 118.
- Biggers J D, Gwatkin R B B & Heyner S 1961 The growth of avian and mammalian tissues on a relatively simple chemically defined medium. *Exp Cell Res* 25: 41.
- Ehrlich H P 1977 The role of epithelial cell migration in wound healing. In *Cholesteatoma: First International Conference* (Iowa City, Iowa) (ed. F McCabe, J Sadé & M Abramson) pp. 69–71. Aesculapius Publishing Company, Birmingham, Ala.
- Duncan R B 1977 Vitamin A: therapy of aural cholesteatoma. In *Cholesteatoma: First International Conference* (Iowa City, Iowa) (ed. B F McCabe, J Sadé & M Abramson) pp. 404–409. Aesculapius Publishing Company, Birmingham, Ala.
- Fell H B & Mellanby E 1953 Metaplasia produced in

- cultures of chick ectoderm by high vitamin A *J Physiol (Lond)* 119: 470
- McLoughlin C M 1961 The importance of mesenchymal factors in the differentiation of chick epidermis II Modification of epidermal differentiation by contact with different types of mesenchyme *J Embryol Exp Morph* 9: 385
- New D A T 1965 Effects of excess vitamin A on cultures of skin from the tail and pads of the embryonic rat and the trunk, tail and pads of the embryonic rabbit *Exp Cell Res* 39: 178
- Saxen L 1966 Effect of tetracycline on osteogenesis *in vitro* *J Exp Zool* 162: 269
- Weiss H & James H 1955 Skin metaplasia *in vitro* induced by brief exposure to vitamin A *Exp Cell Res Suppl* 3: 381

*T. Pahlva*  
*Department of Otolaryngology*  
*University of Helsinki*  
*Finland*

## THE PRE- AND POSTOPERATIVE ENG FINDINGS IN CLINICAL OTOSCLEROSIS AND THE LATE HEARING RESULTS

E Aantaa and E Virolainen

*From the ENT department Turku University Central Hospital Turku Finland*

(Received May 20 1977)

act 125 patients suffering from otosclerosis under  
 1 oto-neurological investigations preoperatively and  
 daily from the second to sixth day postoperatively  
 abnormal ENG in the form of a spontaneous or posi-  
 tional nystagmus directional preponderance or a dimin-  
 ished caloric reaction could be found preoperatively in  
 6 of the patients. No statistically significant difference  
 was seen between patients with or without abnormal ENG findings  
 in the late postoperative hearing results  
 of 3 years in a series of different types of operations  
 nearly half of the patients had postoperative nystagmus  
 On the second day nystagmus beat towards the operated  
 ear in 22.3% of the patients. On the sixth day there was  
 nystagmus only in one third of the patients and it then  
 beat towards the operated ear in only 12.1% of the pa-  
 tients and away from the operated ear in 21.6%. No  
 statistically significant correlation could be found between  
 the nystagmus findings and the late hearing results after  
 3 years observation in this series of different types of  
 operation

The modern surgical techniques for oto-  
 sclerosis, with opening of the oval window  
 covering it with a suitable material and then  
 reconstructing the ossicular chain, always in-  
 volve a certain risk to the labyrinth. Opening  
 of the labyrinth always brings about a reactive  
 disturbance and a serous labyrinthitis, as is  
 well known from the literature. Subjective ver-  
 tigo and spontaneous and positional nystag-  
 mus after operation are the commonest symp-  
 toms and signs of labyrinth irritation, and  
 their severity can depend on the operative  
 technique in question. It is also possible that  
 labyrinthine irritation after operation can in-  
 fluence the late hearing results.

In clinical otosclerosis cochlear involvement  
 often appear to a varying degree. It is as-  
 sumed that otosclerosis can be the cause of  
 this damage to the cochlear part of the inner  
 ear. When otosclerosis damages the cochlea  
 the question naturally arises whether oto-  
 sclerosis can have a disturbing influence on  
 the function of the vestibular organ too.  
 Fewer investigations have been published in  
 this field than concerning the effect on hear-  
 ing.

Hulk & Jongkees (1950), Remecken (1960),  
 Fisch (1965), Meurman et al (1969) and Virol-  
 lainen (1972) have shown different kinds of  
 preoperative vestibular disturbances in 25-  
 30% of patients suffering from otosclerosis.  
 Are these vestibular disturbances correlated  
 with the late hearing results?

## MATERIAL AND METHODS

The material consists of 125 patients who  
 came to their first operation for otosclerosis  
 between the ages of 18 and 64 (mean age 48.2)  
 years. Patients with other ear disease or head  
 trauma and as subjects under medication were  
 excluded. Careful neuro-otological examina-  
 tion and hearing tests were made before opera-  
 tion. Both spontaneous and positional as well  
 as caloric induced nystagmus (modified Hall-  
 pike test) were recorded by an Elema Mingo-  
 graph nystagmograph (a c condenser  
 coupled time constant 5 sec direct ink writ-  
 ing). The spontaneous and positional nystag-  
 mus were recorded in the supine and right and  
 left lateral positions before operation and again  
 daily from the second to the sixth day after

Table I *The numbers of patients in the series*

Type of operation	Number of patients	Mean loss (dB) for 500 1000 2000 cps in operated ears			4000 to 8000 mean (dB)
		Air conduction	Air bone gap		
A Mobilization of the stapes	19 (15.2%)	S D	47.0 12.1	27.9 7.9	51.6 21.9
B Anterior crurotomy and partial laminectomy	44 (35.2%)	S D	48.1 12.5	29.5 9.3	49.3 20.5
C Stapedectomy and polyethylene prosthesis	46 (36.8%)	S D	56.8 13.0	32.6 10.0	64.2 18.7
D Oval window fenestration and interposition	16 (12.8%)	S D	51.0 11.3	30.9 7.4	57.8 20.0
Total	125 (100%)				

operation. The nystagmus findings were considered positive if the speed of the slow phase exceeded seven degrees per second. The hearing of the same patients was also examined 3 years after operation.

The types of operation and the numbers of patients are shown in Table I. Also the mean hearing loss before operation (air conduction and air-bone gap mean dB loss for 500, 1000 and 2000 cps and air conduction mean 4000 to 8000 cps) is shown. In the mobilization series (A), there were 19 (15.2%), in the anterior crurotomy and partial laminectomy series (B) 44 (35.2%), in the stapedectomy and polyethylene prosthesis series 46 (36.8%) and in the oval window fenestration and interposition

series 16 (12.8%) patients. The hearing loss of the contralateral ear was always less than in the ear to be operated. There was no significant difference between each series concerning sex, age or hearing loss.

## RESULTS

Six of 125 (4.8%) patients had preoperative subjective vertigo and 48 (38.4%) had tinnitus.

There was a pathological ENG finding in the form of a spontaneous or positional nystagmus, directional preponderance or diminished caloric reaction in 39 (31.2%) of these patients. Spontaneous or positional nystagmus was recorded in 13 (10.4%) patients. Direc-

Table II *The mean hearing results three years postoperatively in a series of different types of operation*

Type of operation		Air conduction mean (dB)	Air-bone gap (mean loss for 500 1000 2000 cps)	4000 to 8000 mean (dB)
A Mobilization of the stapes		38.7	20.3	43.5
	S D	12.7	12.6	9.5
B Anterior crurotomy and partial laminectomy		24.7	11.0	39.0
	S D	11.1	10.0	11.2
C Stapedectomy and polyethylene prosthesis		34.5	13.7	60.1
	S D	17.8	15.2	19.1
D Oval window fenestration and interposition		25.2	11.2	48.2
	S D	10.7	10.0	17.3

III The numbers of patients in a series of different types of operation and the mean hearing loss three years postoperatively in patients with abnormal and normal ENG before operation

of operation	Air conduction mean (dB)		Air-bone gap mean loss for 500 1 000 2 000 cps		4 000 to 8 000 mean (dB)	
	Abnormal ENG	Normal ENG	Abnormal ENG	Normal ENG	Abnormal ENG	Normal ENG
Mobilization of the stapes	55.0 S D 24.7	36.7 11.9	14.2 18.8	21.1 12.1	60.1 26.5	41.6 6.9
Anterior crurotomy and partial laminectomy	24.6 S D 10.5	24.6 10.8	10.3 10.2	11.4 10.3	36.2 11.6	40.8 10.9
Stapedectomy and polyethylene prosthesis	33.6 S D 20.2	35.5 16.2	16.0 16.4	12.7 15.2	57.5 19.7	61.1 19.2
Oval window fenestration and interposition	26.7 S D 7.5	25.6 14.0	15.2 8.0	7.9 12.4	42.9 8.9	54.1 23.6

normal preponderance was shown by the caloric tests in 14 (11.2%) and diminished caloric response in 19 (15.2%) patients.

Patients who had vertigo in their case history before operation did not show more abnormalities in the ENG than those lacking this symptom. Pathological ENG findings were found to be of a significantly higher degree in the patients complaining of subjective tinnitus than in the other otosclerotic patients. There was no significant preoperative difference in hearing loss between patients with or without abnormalities in ENG. Hearing results after 3 years of operation are shown in Table II.

There are no significant differences in mean air conduction (500, 1000, 2000 cps) in the different series of operations. The air-bone gap is significantly wider in series A (mobilization of the stapes) than in other series.

The mean hearing results (air conduction, mean hearing loss in dB 500, 1000 and 2000 cps) and mean air-bone gap 500, 1000, 2000 cps and mean hearing loss in dB by 4000 to 8000 cps in the subseries with and without preoperative abnormalities in ENG are shown in Table III. There are no statistically significant differences in the hearing results between patients with or without preoperative abnormalities in the ENG. The occurrence and the direction of nystagmus in the whole material

from the second to sixth postoperative day are shown in Table IV. One can see that on the second day there were as many cases with nystagmus beat towards the operated ear as away from it. But subsequently the nystagmus became more infrequent and beat more and more away from the operated ear.

The occurrence of postoperative nystagmus lasting more than 3 days after the operation in the series of different operations is shown in Table V. There are in each series only about 15% patients who had postoperative nystagmus for 3 or more days. When the hearing results 3 years after operation (Table II) were compared with these findings of postoperative

Table IV The direction of nystagmus in the percentage of the whole material (125 subjects) from the second to the sixth day postoperatively

The day after the operation	Nystagmus towards the operated ear (%)	Nystagmus away from the operated ear (%)	No nystagmus (%)
Second	22.3	22.3	55.4
Third	19.8	25.3	54.9
Fourth	16.8	31.5	51.7
Fifth	18.4	24.1	57.5
Sixth	12.2	21.6	66.2

Table V *The postoperative nystagmus*

Type of operation	No nystagmus or nystagmus during less than 3 days postoperatively	Nystagmus during 3 or more days postoperatively	Total
A Mobilization of stapes	16 (84.2%)	3 (15.8%)	19
B Anterior crurotomy and partial laminectomy	38 (86.4%)	6 (13.6%)	44
C Stapedectomy and polyethylene prosthesis	39 (84.8%)	7 (15.2%)	46
D Oval window fenestration and interposition	13 (81.3%)	3 (18.7%)	16
Total	106 (84.8%)	19 (15.2%)	125 (100%)

nystagmus there was no statistically significant difference between the hearing results and postoperative nystagmus in the whole material or in any special case of these operation series

### DISCUSSION

On the basis of these results one can see that objectively verified vestibular disturbances very often appear in patients suffering from otosclerosis. The changes were noticed in 31 % of patients. This is about the same rate as in earlier investigations (Hulk & Jongkees, 1950, Reinecken, 1960, Fisch, 1965, Meurman et al., 1969). Patients who had preoperative tinnitus had more abnormalities in their ENG recordings than others. The reason for both signs could be an otosclerotic vascular change or a biochemical change of the inner ear fluids or some other reason not yet known. However, the preoperative hearing or the hearing 3 years after operation was about the same in patients with or without tinnitus, which could also be a sign of damage to the inner ear. One had also expected that in those patients who had abnormalities in their preoperative ENG, the hearing results would be worse than in those without any abnormalities. Three years after operation one could not detect any differences between these series. Earlier investigations have shown that there is a correlation between preoperative vestibular dis-

turbances and high tone loss at 4 000 and 8 000 cps (Meurman et al., 1969, Virolainen 1972, Fisch, 1965). The results of this investigation are not in agreement with these findings. On the second day after the operation in our cases, nystagmus was recorded in 44.6 % of patients. In half of the patients with nystagmus, beating was towards the operated ear and, in the other half away from it. Nystagmus which beats towards the operated ear is a sign of a mild labyrinthine disturbance, whereas nystagmus which beats away from the operated ear is a sign of a greater disturbance. This was also the case in our investigation. On the sixth day there was nystagmus only in 33.8 % of patients, but in two thirds of them the nystagmus beat away from the operated ear. This trend continued on each subsequent day. Nystagmus which beats towards the operated ear disappears but nystagmus which beats away from the operated ear will continue. This result differs from Ivstam's (1962) and Fisch's (1965) results, who found that the most frequent type of nystagmus was directed towards the operated ear but their operative techniques were different. Fortunately nystagmus findings in no way correlate with the final hearing results.

### ZUSAMMENFASSUNG

Es wurden 125 Patienten mit Otosklerose an der HNC Abteilung der Universität Turku Finnland prä- und postoperativ untersucht. Von diesen ze-

Veränderungen in Elektronystagmographie in Form spontanem oder Lagenystagmus directional pre oder erniedrigter kalorischer Erregbarkeit. Die Hälfte der Patienten hatten postoperativen Nystagmus. Während des zweiten Tages schlug Nystagmus nach der Seite des operierten Ohres bei 22,3% der Patienten. Während des sechsten Tages gab es Nystagmus bei einem Drittel der Patienten und Nystagmus schlug nach der Seite des operierten Ohres bei 1,2% der Patienten und nach der Seite des unoperierten Ohres bei 1,2% der Patienten. Es gab keine Korrelationen mit Findungen und späteren Gehörresultaten nach drei

- Hulk J & Jongkees J B W 1950 Vestibular examination in cases of otosclerosis *J Laryngol Otol* 64 126  
 Ivstam B 1962 Cochlear and vestibular disturbances after stapediolysis *Acta Otolaryngol* (Stockh) 55 151  
 Meurman O H Aantaa E & Virolainen E 1969 Vestibular disturbances in clinical otosclerosis *Arch Otolaryngol* 90 756  
 Reinecken R 1960 Vestibularuntersuchungen nach Operationen an ovalen Fenster *Z Laryng Otol* 39 432  
 Virolainen E 1972 Vestibular disturbances in clinical otosclerosis *Acta Otolaryngol* (Stockh) Suppl 306

## REFERENCES

h U 1965 Vestibuläre Symptome vor und nach Stapedectomy *Acta Otolaryngol* (Stockh) 60 515

E Aantaa  
 ENT Department  
 TYKS  
 SF 20500 Turku 52  
 Finland



## SKULL DISTORTION OF BONE CONDUCTED SIGNALS

S D Arlinger, P Kylen and H Hellqvist

*From the Departments of Audiology and Otolaryngology University Hospital Linköping Sweden*

(Received July 27 1977)

**Abstract** A previously essentially unknown type of distortion of bone conduction (BC) signal has been studied on the skulls of four human cadavers. The method was based on a miniature accelerometer rigidly attached to the cranial bone converting the skull vibration close to the cochlea into an electrical signal which was analysed with regard to harmonic distortion. The BC signals pure tones were presented by means of a high quality vibrator. The distortion was found to be limited to the lower audiometric frequencies with a maximum around 500 Hz and of such a degree as to be able to significantly influence the results of BC audiometry. The distortion is probably caused by nonlinear mechanical properties of the human skull.

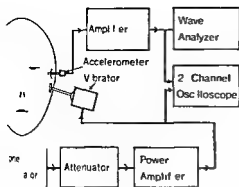
Pure tone audiometry by means of bone conducted (BC) signals is an established method in clinical audiology, which permits evaluation of the sensory neural function of the auditory system.

However, BC audiometry has several limitations, some of which are determined by the equipment used, particularly the vibrator. Normally, electromagnetic vibrators, primarily intended for use in BC hearing aids, are employed since they are easy to apply due to their small size. Related to these small dimensions, however, the frequency response of these vibrators is often poor and shows mechanical resonance in the range 200-600 Hz. The harmonic distortion produced may influence hearing threshold measurements—at least at 250 and 500 Hz (Dirks & Kamm 1975). This nonlinear distortion of the vibrator increases with signal level, which thus contradicts the clinical requirement for higher available signal levels for BC audiometry.

In an earlier investigation concerning BC stimulation for electrocochleography (Arlinger

& Kylen, 1977), the ability of various types of vibrator to convert brief or transient electrical signals linearly to mechanical motion was studied. The vibrators were placed on various locations on the skulls of human cadavers and the skull vibration patterns were recorded by means of a miniature accelerometer, rigidly attached to the promontory in the middle ear. A pronounced nonlinear distortion was found in the accelerometer output signal using tonal bursts at 500 Hz but not at higher frequencies. This distortion was present for all types of vibrators and all vibrator sites used. It was consequently suspected that this distortion was not due to the nonlinear behaviour of the vibrator but might be determined by nonlinear properties of the human skull.

Kinkadee (1959), when exploring vibration patterns of human skulls excited by a vibrator, also noted that the accelerometer signal often contained components of low frequencies superimposed on a signal of the same low frequency that was fed to the vibrator. He assumed that this phenomenon was caused by harmonics of the low frequency vibration at or close to the first resonance frequency of the skull around 1800 Hz. Kinkadee et al (1976) also came across the same distortion. In a series of cancellation experiments, i.e. psychoacoustic experiments where the level and phase of an air conducted pure tone was adjusted to cancel the auditory sensation of a bone conducted pure tone, he found that for frequencies below 2 kHz the point of cancellation, i.e. when the mental component disappeared ■



Block diagram of experimental equipment

remained. The relative level of this was considerably higher than what could possibly be produced by the vibrator itself. This distortion was amplitude dependent. They propose two possible explanations: "near properties in the coupling between vibrator and skull, and the basic asymmetry of the cochlear response". Thus the findings in these studies point to a type of nonlinear distortion which may occur during stimulation in the low frequency region which is probably due to anatomical and physiological properties of the human skull. Since this distortion was of such a magnitude that it could influence the results of BC audiometry, a more detailed investigation seemed imperative.

## METHOD

The intact skulls of four human cadavers were investigated. During the experiments the cadaver was placed on a wooden head rest covered by a 1 cm thick layer of foam. A miniature accelerometer (Bruel & Kjaer type 8303, 3.5 g) was used as a vibration pick up. One end of a 35 mm long rod (1.8 mm diam, 1 g weight) was attached to the accelerometer. A small hole was drilled into the exposed middle ear. In this hole the end of the rod was placed and firmly fixed to the bone by means of cranial cement. The signal from the accelerometer was amplified (Bruel & Kjaer 3501) and fed to a wave analyser (Radiometer 31) for the harmonic analysis (see Fig. 1). The wave analyser was used with its bandwidth set to its smallest range,  $\pm 1$  dB  $\pm 25$  Hz at  $-50$  dB.

Sinusoidal signals were generated by a tone generator (Hewlett Packard 241 A) attenuated (Hewlett Packard 340 D loaded by a 600 ohm resistor) and fed to a 15 W audio power amplifier (Vingtor K 2 T). The output of which was connected to the vibrator (Bruel & Kjaer 4810 Mini Shaker). This vibrator has a distortion of less than 1% in the frequency range 150–1000 Hz at 10 g acceleration. This acceleration corresponds to a level of approximately 70 dB above normal threshold of hearing for bone conduction (Khanna et al. 1976).

The input signal to the vibrator as well as the amplified accelerometer output signal were monitored on a 2 channel oscilloscope (Tektronix 561 A).

The Mini Shaker was fitted with a special adaptor made by a 60 mm long 6 mm diam. aluminum rod with a plane circular tip surface having an area of approx. 1.75 cm<sup>2</sup>. A special holder was used for the vibrator by means of which the vibrator's position, direction and static application force could be adjusted. A static force of approx. 5 N was used in the experiments.

The vibration levels used in the study were determined by means of calibration on a Bruel & Kjaer 4910 Artificial mastoid with reference to the bone conduction threshold data presented by Flottorp (1972). Stimulation levels are thus given in dB HL.

The harmonic distortion of the entire stimulation apparatus was measured by placing the vibrator with its adaptor on the artificial mastoid, the output of which was amplified (Bruel & Kjaer 2603) and analysed with regard to harmonic components (Radiometer FRA3). The second harmonic at maximum signal levels used was  $-31$  dB re the fundamental at 250 Hz/60 dB HL,  $-35$  dB at 400 Hz/65 dB HL and  $-35$  dB at 750 Hz/70 dB HL. At lower levels or at higher frequencies the second harmonic was 40 dB or more below the level of the fundamental. All higher harmonics were always more than 45 dB below the fundamental.

Since this type of skull distortion evidently only occurred in the lower frequency range, the experiments were limited to the frequencies 250, 400, 750, 1000 and 1500 Hz. Maximum stimulation levels of 60–70 dB HL were used, comparable to what is found in commercially available audiometers. The main experiments concerned the vibrator placed on the ipsilateral mastoid process, but also forehead placement was studied.

## RESULTS

A common way of analysing nonlinear distortion of a signal transmission link is to feed the link with a sinusoidal input signal of frequency  $f_0$ . Nonlinear transmission properties in the link causes harmonic distortion, i.e. the output signal will no longer be a pure sinusoid but contain, in addition to the fundamental component (at frequency  $f_0$ ), also various harmonics at frequencies  $N \times f_0$ , where  $N$  is an integer. The degree of harmonic distortion is

## SKULL DISTORTION OF BONE CONDUCTED SIGNALS

S D Arlinger, P Kylén and H Hellqvist

*From the Departments of Audiology and Otolaryngology, University Hospital, Linköping, Sweden*

(Received July 27 1977)

**Abstract** A previously essentially unknown type of distortion of bone conduction (BC) signal has been studied on the skulls of four human cadavers. The method was based on a miniature accelerometer, rigidly attached to the cranial bone converting the skull vibration close to the cochlea into an electrical signal which was analysed with regard to harmonic distortion. The BC signals (pure tones) were presented by means of a high quality vibrator. The distortion was found to be limited to the lower audiometric frequencies with a maximum around 500 Hz and of such a degree as to be able to significantly influence the results of BC audiometry. The distortion is probably caused by nonlinear mechanical properties of the human skull.

Pure tone audiometry by means of bone conducted (BC) signals is an established method in clinical audiology, which permits evaluation of the sensory neural function of the auditory system.

However, BC audiometry has several limitations, some of which are determined by the equipment used, particularly the vibrator. Normally, electromagnetic vibrators, primarily intended for use in BC hearing aids, are employed since they are easy to apply due to their small size. Related to these small dimensions, however, the frequency response of these vibrators is often poor and shows mechanical resonance in the range 200-600 Hz. The harmonic distortion produced may influence hearing threshold measurements—at least at 250 and 500 Hz (Dirks & Kamm, 1975). This nonlinear distortion of the vibrator increases with signal level which thus contradicts the clinical requirement for higher available signal levels for BC audiometry.

In an earlier investigation concerning BC stimulation for electrocochleography (Arlinger

& Kylén 1977), the ability of various types of vibrator to convert brief or transient electrical signals linearly to mechanical motion was studied. The vibrators were placed on various locations on the skulls of human cadavers and the skull vibration patterns were recorded by means of a miniature accelerometer, rigidly attached to the promontory in the middle ear. A pronounced nonlinear distortion was found in the accelerometer output signal using tonbursts at 500 Hz but not at higher frequencies. This distortion was present for all types of vibrators and all vibrator sites used. It was consequently suspected that this distortion was not due to the nonlinear behaviour of the vibrator but might be determined by nonlinear properties of the human skull.

Kinkadee (1959) when exploring vibration patterns of human skulls excited by a small vibrator, also noted that the accelerometer signal often contained components of higher frequencies superimposed on a signal of the same low frequency that was fed to the vibrator. He assumed that this phenomenon was caused by harmonics of the low frequency vibration at or close to the first resonance frequency of the skull, around 1800 Hz. Khan et al (1976) also came across the same type of distortion. In a series of cancellation experiments, i.e. psychoacoustic experiments where the level and phase of an air conducted pure tone was adjusted to cancel the auditory sensation of a bone conducted pure tone, they found that for frequencies below 2 kHz the point of cancellation i.e. when the fundamental component disappeared a second



Fig 3 Levels of the second-fifth harmonics as functions of frequency in the range 250-750 Hz. Input voltage to vibrator corresponded to a BC signal level of approx. 65 dB HL at 500 Hz. Mean values from three skulls

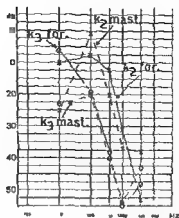


Fig 4 Levels of the second and third harmonics as functions of frequency with vibrator placed on the mastoid process and on the forehead. BC signal level 60 dB HL. Mean values of four skulls

50-750 Hz was studied in 50 Hz intervals on three of the four skulls. An input voltage to the vibrator corresponding to approx. 65 dB HL at 00 Hz was used. The mean levels of the second-fifth harmonics are shown in Fig 3. The dominating second and third harmonics both have clear maxima at or very near 500 Hz.

On all four skulls the vibrator was also placed on the forehead to examine the influence of vibrator placement. Fig 4 shows the mean levels of the second and third harmonics for forehead and mastoid placements at 60 dB HL (data for 500 Hz obtained by linear interpolation from results at 55 and 65 dB HL). Only at 250 Hz a clear difference of any importance is seen. At this frequency the harmonic distortion, measured in the accelerometer output signal, was considerably larger when the vibrator was placed on the forehead than when it was placed on the mastoid.

Furthermore, it was found that the vibrator was on average 20 dB less efficient in transferring the fundamental frequency of 250 Hz when placed on the forehead than when placed on the mastoid, as seen in the accelerometer output signal level at this frequency. At the higher frequencies this difference in efficiency between the two vibrator placements was

smaller, the mean difference being 7 dB at 500 Hz, 4 dB at 750 Hz, 6 dB at 1 kHz and 0 dB at 1.5 kHz with the forehead consistently offering the poorest transmission to the skull.

## DISCUSSION

The results clearly confirm the previously noted existence of a serious distortion of bone conducted signals in the lower range of audiometric frequencies (Arlinger & Kylen, 1977).

The distortion of the electronic equipment, including the vibrator as used in the present study, was significantly lower than the results obtained as presented in Figs 2-4. The fixation of the accelerometer to the brass rod and of the rod to the cortical bone was tested manually both before and after measurements and was always found to be very rigid. In fact, a considerable force had normally to be applied to the rod to remove it after the completion of the measurements. It may thus be concluded that no measurement artefacts due to the equipment and its use can explain the results. The conclusion is further supported by the previously quoted findings of similar nature in other studies (Kinkade, 1959; Khanna et al, 1976).

The distortion obtained thus must be caused by nonlinear properties of the biological tissues involved. Khanna et al (1976) suggested that the soft tissues (skin and underlying tissues) may not behave as a perfectly elastic coupling agent. However, this suggestion is contradicted by the results of von Békésy (1960), who tested the linearity of vibration transmission through the skin at 400 Hz. Using the web of the skin between the index finger and the thumb, placed between a vibrator and a vibration pick up attached to a large mass, he varied the vibration amplitude from  $10^{-3}$  to  $10^{-10}$  cm and found no evidence for nonlinear behaviour of the skin. Further, in our previous study (Arlinger & Kylen, 1977) the vibrator was also placed directly on the exposed bone surface of the mastoid process after removing the soft tissues. Although no quantitative measurements were performed, a significant distortion of the same order of magnitude as that found in the present study was seen in the accelerometer output signal displayed on an oscilloscope.

One possible explanation for the high harmonic levels obtained might be that pronounced resonance phenomena in the skull give rise to a high amplification of weak harmonics present in the vibrator motion.

Kirikae (1959) performed detailed mapping studies of the vibrational patterns of human skulls. He found the first resonance frequency of the skulls to occur at 1700–1800 Hz. One would then expect to find the dominant harmonic components in this frequency range. This is not the case, however. As seen in Figs 2 and 3, at the signal frequencies of particular interest in this respect, 250 and 500 Hz, the distortion is dominated by the third harmonic (750 Hz) at 250 Hz and by the second harmonic (1 kHz) at 500 Hz, i.e. by harmonics at clearly lower frequencies than the first resonance of the skull.

The origin of the BC signal distortion is consequently most likely to be found in the bone tissue of the skull. It is a well established fact that bone tissue has elastic as well as visco-

elastic and plastic properties (Evans 1971) and thus may show a nonlinear relation between the driving vibrational force and vibration amplitude obtained.

The distortion was found to be most pronounced at 500 Hz. The reason for this is not known but may be related to changes in the vibration pattern as the frequency is increased. Both Kirikae (1959) and von Békésy (1969) found the skull to behave as a rigid body at very low frequencies (200–250 Hz). At 500–800 Hz one circular nodal line was found while at 1000 Hz the skull vibrates with two nodal lines. At higher frequencies the pattern becomes increasingly complex. The fact that the viscoelastic and plastic properties of tissue depend on the rate of mechanical loading, i.e. on vibration frequency, may also be an explanation for this finding.

The distortion of BC signals thus seems originate from nonlinear mechanical properties of the human skull that can hardly be eliminated. The practical consequence of these findings is the risk of measurement error in BC tone audiometry. This risk is due to the possibility that a patient may respond to a stimulus tone which is below his threshold hearing, while the fundamental component of the stimulus tone is still below his threshold hearing, thus giving rise to a false air gap. This risk is particularly evident for subjects with sensorineural hearing loss of the low frequency type, e.g. Mb Ménière, but also for flat losses.

The existing IEC publication concerning requirements on diagnostic hearing aids (IEC 177), requires that each harmonic component should be at least 30 dB below the level of the fundamental component for air conduction. In a study on detection of amplitude distortion, Gabriellson et al (1976) concluded that normal hearing subjects rarely show more than 2.5% quadratic and 1.25% cubic distortion, i.e. when the second and third harmonics are 32 and 38 dB below the level of the fundamental, respectively. Based on these results, the maximum permissible harmonic level of 30 dB re the level of the fundamental for BC

y seems a realistic minimum requirement

Applying this requirement to the present results makes it possible to define for each pure tone frequency a critical level, above which skull distortion reaches such a degree that it may cause measurement errors. The critical levels thus obtained from the present results are the following: 50 dB HL at 250 Hz, 25 dB HL at 500 Hz, 50 dB HL at 750 Hz, 65 dB HL at 1 and 1.5 kHz.

Hearing thresholds for bone conduction, at higher levels than these, must be evaluated with care. Because of harmonic distortion in the bone vibrators commonly used in audiometry, BC measurement errors may occur at even lower signal levels, particularly at 250 Hz.

## CONCLUSIONS

1 A previously essentially unnoticed type of distortion of bone conduction signals has been investigated on the skulls of four human cadavers. The distortion seems to be caused by nonlinear mechanical properties of the bone tissue of the skull.

2 The distortion mainly concerns signals in the low frequency region, with a maximum around 500 Hz.

3 The distortion reaches such a magnitude as to be able to cause significant measurement errors in bone conduction tone audiometry.

4 Frequency dependent critical signal levels have been presented above which the distortion has been found to be significant. Hearing thresholds for bone conduction obtained above these critical levels must be evaluated very carefully with regard to possible measurement error due to the skull distortion.

## ACKNOWLEDGEMENT

This investigation was financed in part through the Linköping University Program Budget.

## ZUSAMMENFASSUNG

Eine früher im wesentlichen unbekannte Verzerrung von Knochenleitungssignalen ist mit Hilfe von Vibrationsmessungen an intakten Leichenschädeln studiert worden. Sinustöne wurden über einen guten Vibrationsgeber präsentiert. Die Verzerrung war zum tieferen audiometrischen Frequenzbereich begrenzt mit einem Maximum bei 500 Hz und so groß daß sie die Resultate von Knochenleitungsaudiometrie beeinflussen mochte. Die Verzerrung ist wahrscheinlich durch unlineare Eigenschaften des menschlichen Schädels verursacht.

## REFERENCES

- von Watzky J  
McGraw Hill New York
- Durks H D & Kamm C 1975 Bone vibrator measurements: physical characteristics and behavioral aspects. *Acta Otolaryngol* 83: 12-24.
- Chen C  
Report  
Institutet  
Stockholm Sweden
- IEC Publication 177 1965 *Pure Tone Audiometers for General Diagnostic Purposes*. International Electrotechnical Commission Geneva Switzerland.
- Khanna S M, Tonndorf J & Queller J F 1976 Mechanical parameters of hearing by bone conduction. *J Acoust Soc Am* 60: 139.
- S D Arlinger MD  
Dept of Audiology  
University Hospital  
S 581 65 Linköping  
Sweden
- ndamen  
laryngeal

## HEARING LOSS RESULTING FROM PERILYMPH FISTULA

### *A Presentation of Two Cases*

Terje Gundersen and Otto Inge Molvær

*From the ENT Department Regionsykehuset Trondheim Norway*

(Received September 4 1977)

**Abstract** Two cases of perilymph fistula in the oval window are presented. In such cases hearing loss may be severe and vertigo may or may not be present. Early surgical intervention is recommended but one should not hesitate to explore a suspected ear, even if considerable time has elapsed.

Many cases of suspected and verified perilymph fistula, spontaneous and traumatic, have been reported (Edmonds, 1973, Edmonds et al., 1973, Farmer & Thomas, 1976, Fee, 1968, Freeman & Edmonds, 1972, Freeman et al., 1974, Goodhill, 1971, Goodhill, 1972, Goodhill et al., 1973, Healy et al., 1974, Mulvaney & Montandon, 1974, Pullen, 1972, Stroud & Calcaterra, 1970).

The causal mechanism may be explosive or implosive (Edmonds, 1973, Farmer & Thomas, 1976, Goodhill, 1971, Goodhill et al., 1973). Explosive forces are initiated by all manoeuvres raising central venous pressure and cerebral spinal fluid pressure, which is then mediated to the perilymph, mainly through the ductus perilymphaticus. An implosive force is raised air pressure reaching the middle ear through the Eustachian tube during the Valsalva manoeuvre.

This paper will deal mainly with hearing loss resulting from perilymph fistula. The audiograms were recorded according to the ISO Standard of 1964.

## MATERIALS AND METHODS

### *Case 1*

A 38-year old woman had a right sided stapedectomy (piston technique) performed else-

where because of otosclerosis. Postoperatively she experienced vertigo and nausea which was worst on the fourth postoperative day. After 2 more days her symptoms gradually subsided, but hearing tests showed the operated ear to be deaf. The patient was told that the reason was a postoperative virus infection. She was discharged from hospital after 16 days and was ill for another month. She was still much troubled by whirling tinnitus and with poor balance, especially early in the day, and she experienced vertigo every time she tried to clean her ear. In addition she felt as though her right eye deviated involuntarily. Since the hearing on the left side was also impaired, she obtained a hearing aid. The vertigo subsided gradually but reappeared at once if she turned abruptly or blew her nose.

Three months after the operation she was examined here as an out patient. The audiograms were confusing and we could not be sure whether or not her right ear was stone deaf (Fig. 1). Tuning fork tests were inconclusive, and the speech audiogram showed 0% score. We feared that her ear was completely deaf and that her vertigo was caused by a perilymph fistula in the oval window. Accordingly she was hospitalized one month later and a right sided exploratory tympanotomy was performed under local anaesthesia. The oval window was found to be completely open without any trace of the stapes footplate. The teflon piston was pointing towards the edge of the window and was replaced by a steel wire fat prosthesis. At once the patient declared

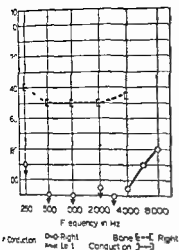


Fig 1 Initial audiogram from case 1 3 months after the stapedectomy. O Air conduction masked 95-110 dB. Bone conduction masked 105 dB.

the noise in the room had altered in character. Two days postoperatively a pure tone audiogram showed relatively good hearing in the ear and in speech audiometry she scored 80% at 100 dB SPL. Four days later the hearing was even better, with a 40 dB mean loss in the speech frequencies, and a 100% score at 90 dB by speech audiometry (Fig 2). Her vertigo subsided and she left hospital 2 weeks after the operation.

The hearing fluctuated, however, and since a conductive component in the loss seemed to remain she was readmitted about 2 months after the operation. The ear was explored a second time but no explanation for the mixed fluctuating hearing loss was found. The prosthesis was in place and everything seemed to be in order. She left hospital on the fifth postoperative day and her hearing has continued to fluctuate. The last audiogram showed a marked deterioration compared with our best recording.

## Case 2

A 25-year old fighter pilot skin dived to the bottom of a 4 m (13 ft) deep swimming pool. During flying he had never had any difficulty in clearing his ears and during descent he never felt pain even though he did nothing

active to equalize the pressure to his middle ears. At the bottom of the pool he suddenly felt as if his right ear had become plugged. He also experienced right-sided tinnitus but no vertigo. The sensation of a plug in the ear and the tinnitus continued after the dive but he still experienced no vertigo in spite of flying a light aircraft afterwards. Since the ear symptoms did not clear he was referred to one of us by the military physician 2 days later. Except for slight injection of the nasal and pharyngeal mucosa the ENT examination disclosed nothing abnormal. A pure tone audiogram, however, showed a marked sensorineural hearing loss on the right side (Fig 3). Consequently he was put on the sick list and forbidden all physical effort. The following week his hearing had not improved satisfactorily and he was therefore hospitalized on bed rest for another week. During this time his hearing improved dramatically (Fig 4) but he still had a loss of 70 dB at 4 kHz which might have interfered seriously with his planned career as a pilot. Therefore 2 weeks after the accident we explored his right middle ear and found a leakage of perilymph through the annular ligament in the inferior part of the oval window which was then covered with Spongostan. The patient left hospital 8 days postoperatively and has since

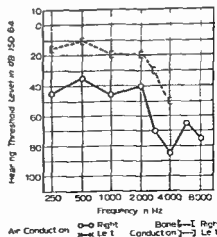


Fig 2 Best audiogram from case 1 6 days after closure of the fistula.



had no trouble with his hearing. The last audiogram, recorded 21 months after the accident, shows near normal hearing also at the 4 kHz frequency (Fig. 4). The patient maintains that he had a 'notch' in the high frequency range of his audiogram on the right side before the accident, but we have not been able to obtain that audiogram for comparison.

## DISCUSSION

A definite preoperative diagnosis of perilymph fistula is difficult or impossible. Most authors therefore recommend surgical exploration of the ear if a fistula is suspected (Edmonds, 1973; Edmonds et al., 1973; Farmer & Thomas, 1976; Fee, 1968; Freeman et al., 1974; Goodhill, 1971; Goodhill, 1972; Goodhill et al., 1973; Healy et al., 1974; McCormick et al., 1976; Mulvaney & Montandon, 1974; Pullen, 1972; Stroud & Calcierterra, 1970). We fully agree with this procedure. Perhaps one should allow a couple of days of complete bed rest in case spontaneous closure occurs. If vertigo subsides and hearing is restored one may postpone surgical intervention. The patient must be strongly advised to avoid the Valsalva manoeuvre, vigorous nose blowing and, if possible, sneezing pre and postoperatively. It is equally important to avoid any other manoeuvre

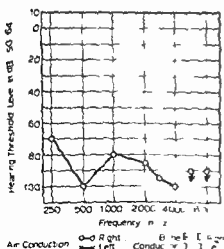


Fig. 3 First audiogram from case 2 7 days after the accident. As hearing loss was purely sensorineural the bone conduction curve is omitted for clarity.

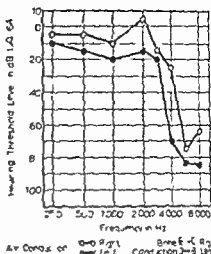


Fig. 4 ● Immediate preoperative audiogram from case 2, 2 weeks after the accident. ○ Most recent follow-up audiogram from case 2 21 months after the accident. As hearing loss was purely sensorineural the bone conduction curves are omitted for clarity.

tending to raise the central venous and cerebrospinal fluid pressure such as heaving or straining at stool. Should any of these manoeuvres occur in the postoperative phase the fistula may re-open and another operation may become necessary (Fee, 1968; Pullen, 1972).

Our second patient was strongly discouraged from further diving but we found no reason to interfere with his flying career. To experience in flight a sudden pressure change corresponding to a dive to a depth of 4 m (13 ft) of water would mean complete loss of cabin pressure at a 24 000 ft altitude, a very unlikely event.

Hearing loss and vertigo from perilymph fistula seems to be a growing problem among divers, as reflected in the literature. Diving mammals seem to escape the problem through adapted anatomical peculiarities (McCormick et al., 1976).

There are two points about these cases worth stressing.

1. Perilymph fistula may lead to profound hearing loss even greater than in cases with destruction of both the incus and the suprastructures of stapes or with complete fixation of the stapes footplate. In the first pa-

sent we initially feared that the ear might be completely deaf

2 It seems as though leakage of perilymph may exist for a long time without the patient developing infective labyrinthitis. This view is supported by our observations during several stapedectomies where stapedolysis had been performed years earlier and where leakage of perilymph through the oval window was still present at the time of our operation. None of those patients showed any sign of past or present infective labyrinthitis nor did the history suggest it. Nevertheless, we suggest not to hesitate too long before exploring an ear suspected of perilymph fistula, since much may be gained and little lost with this procedure.

## ZUSAMMENFASSUNG

Zwei Fälle mit perilymphatischen Fistelbildungen am

empfehlen. Eben in Fällen, wo der Zustand lange ge-  
dauert hat, ist eine abwartende Haltung nicht zu emp-  
fehlen.

## REFERENCES

- Edmonds C 1973 Round window rupture in diving  
*Fo i Armed cin* 9 (3) 404-405  
Edmonds C, Freeman P, Thomas M, Tonkin J &  
Backwood F A 1973 *Otological Aspects of Diving*

- pp 47-53 64-81 Australasian Medical Publ Co  
Ltd New South Wales  
Farmer jr J C & Thomas W C 1976 Ear and sinus  
problems in diving Pp 109-133 in *Diving Medicine*  
(ed R H Strauss) Grune & Stratton New York  
San Francisco London  
Fee G A 1968 Traumatic perilymphatic fistulas  
*Arch Otolaryngol* 88 477-480  
Freeman P & Edmonds C 1972 Inner ear barotrauma  
*Arch Otolaryngol* 95 556-563  
Freeman P, Tonkin J & Edmonds C 1974 Rupture  
of the round window membrane in inner ear baro-  
trauma *Arch Otolaryngol* 99 437-447  
Goodhill V 1971 Sudden deafness and round window  
rupture *Laryngoscope* 81 1462-1474  
— 1972 Inner ear barotrauma *Arch Otolaryngol* 95  
588  
Goodhill V, Brockman S J, Harris I & Hantz O  
1973 Sudden deafness and labyrinthine window  
ruptures *Ann Otol Rhinol Laryngol* 82 2-17  
Healy G B, Strong M M & Sampogna D 1974  
Ataxia, vertigo and hearing loss: A result of rupture  
of inner ear window *Arch Otolaryngol* 100 130-135  
McCormick J G, Wever E G, Hamill J A & Miller  
H M 1976 Anatomical and physiological adaptations  
of marine mammals for the prevention of diving in-  
duced middle ear barotrauma and round window  
fistula *Undersea Biomed Res* 3 (1) A48-A49  
Mulvaney T J & Montandon P B 1974 Traumatic  
oval window fistula with cerebrospinal fluid gusher  
*Ann Otol Rhinol Laryngol* 83 414-415  
Pullen F W 1972 Round window membrane rupture  
a cause of sudden deafness *Trans Am Acad Ophthal-  
mol Otolaryngol* 76 1444-1450  
Stroud M H & Calaterra T C 1970 Spontaneous  
perilymph fistulas *Laryngoscope* 80 479-487

Prof T Gundersen M D  
ENT Department  
Regionsykehuset  
Trondheim  
Norway

## SPEECH AND PURE TONE AUDIOMETRY AS A SCREEN FOR EXAGGERATED HEARING LOSS IN INDUSTRIAL CLAIMS

P. W. Alberti,<sup>1</sup> P. P. Morgan<sup>2</sup> and I. Czuba<sup>2</sup>*From the <sup>1</sup>Department of Otolaryngology and the Mount Sinai Hospital Toronto and the <sup>2</sup>Department of Preventive Medicine and Biostatistics University of Toronto Canada*

(Received December 6 1976)

**Abstract** We examined 596 patients referred with possible noise induced hearing loss by conventional and cortical evoked response audiometry. Discrepancies between the two tests identified 121 (20.3%) as exaggerating their hearing loss. We then studied the validity of simple conventional tests which would be available in primary diagnostic facilities in screening for the exaggerators we had identified. By selecting those whose puretone average (for 0.5 and 1 kHz) was more than 10 dB different from the speech reception threshold or whose initial puretone threshold at 0.5 kHz was 40 dB or greater, we identified 112 (93%) of the exaggerators, at the cost of additional examination of 209 (35% of the total) false positive. However this screening means that 46% of the claimants could be evaluated completely in a primary diagnostic facility and only a small fraction of the exaggerators should be overlooked.

Hearing loss caused by exposure to industrial noise is one of the major public health issues concerning Otolaryngologists today. In the Province of Ontario, Canada, occupational hearing loss first became compensable through The Workmen's Compensation Board—essentially an industrial insurance Board—in 1948 and, after a slow start, claims have nearly doubled in each of the last three years. There were about 900 claims in 1974, 1500 in 1975, and 2500 are expected in 1976. The proportion actually receiving compensation has remained constant.

In Ontario there is an uneven distribution of population and noisy industry. 80% of claims currently come from more than 300 kilometers north of Toronto, although only 20% of the population live so far away. To compound

matters, almost all of the elaborate testing facilities are in the southern towns.

Whenever compensation is directly related to severity of disease, great care must be taken in the accurate quantification of the disorder. Hearing loss is no exception. It is generally accepted that the rate of exaggerated hearing loss in industrial claims is between 15 and 20% and the degree of exaggeration is usually more than 15–20 dB, almost always on top of genuine coexisting hearing loss. However, whilst these figures do not seem great when translated into compensation awards, they represent a considerable sum of money at stake. Previous work from our laboratory (Alberti & LeBlanc, 1976) has shown that the average lifetime saving per claim per exaggerator is approximately \$5000. Of the anticipated 2500 claimants in 1976 about 10% will be exaggerating, and if undetected will over their lifetime cost an additional \$1 million; their detection could save almost \$90 000 annually.

The Compensation Board have, up to now, requested that most claimants travel to a major centre in order that accurate evaluation can be undertaken. This is a significant disturbance and expensive dislocation of people, the justification for which lies in the need to detect exaggerators, compounded by the

sence of sophisticated testing facilities in the northern part of the Province

The purpose of this communication is to describe investigations we have undertaken to attempt to identify potential exaggerators by means of simple hearing tests thus separating those who should be tested in sophisticated facilities from those whose hearing has been accurately quantified by simple tests. This goal is a common one in many countries for few have adequate facilities for full tests of all workers; it is necessary to determine who needs them.

## MATERIAL

In our laboratory we have seen about 1500 claimants in the past 5 years and kept careful records of our findings which have been computer stored for further study. Our goal was to see if examination of puretone thresholds alone or comparison of puretone thresholds and speech reception thresholds would have provided an appropriate screening test for exaggerators.

A group of patients was chosen for this study in whom we have accurate puretone thresholds to act as a base. They were then compared with a group of exaggerators in whom we believe we were successful in establishing an accurate final puretone threshold. Both groups had cortical electrical response audiometry (ERA) undertaken in addition to other tests. We have used ERA in neurologically normal adult military and industrial hearing loss claims since early in 1969 (Albert 1970, Albert et al 1974) for a variety of reasons: sometimes on the total population, sometimes sampling, and sometimes only when suspicious. Evoked response and routine audiometry has always been performed and interpreted by a different tester. If the results differed tests were repeated. In all instances the figures taken as the final true threshold was the result of at least two different tests agreeing, i.e. conventional pure

tone threshold and evoked response matched or evoked response and speech reception threshold matched.

In an earlier unpublished study in non-exaggerating industrial patients puretone threshold results and speech reception threshold had been compared utilizing the recorded Los Angeles Foundation W1 and W2 tests—in an attempt to verify the Fletcher formula for our patients. In the sloping audiogram typical of noise induced hearing loss there was a closer relationship between the SRT and puretone average hearing at 500 and 1000 Hz than there was among the puretone averages at 500, 1000 and 2000 Hz, although the puretone average at 500 and 1000 Hz was 2½ dB ( $\pm 7$  dB) better than the speech reception threshold; we feel that SRT can be used as a reliable estimator of lower frequency puretone thresholds.

Patients who initially exaggerated their hearing loss were identified by means of ERA, tester suspicion and discrepancies between the various hearing tests. Exaggerated hearing loss was defined as a greater than 10 dB hearing discrepancy between the summed average hearing loss of 500 and 1000 Hz in both ears on the initial test results in our laboratory and the final ultimately accepted threshold. This final threshold was usually arrived at after ERA's had shown the initial threshold to be 10 dB or more too high.

## RESULTS

The entire population who had both conventional and evoked response tests undertaken consisted of 596 patients, 121 of whom (or 20.3%) had exaggerated hearing loss.

### (a) SRT puretone threshold comparisons

Detailed frequency distribution studies were made of the conventional puretone threshold/SRT relationships. Preliminary inspection of the results suggested that a reasonable cutoff point was an agreement within 10 dB or less to

Table I Puretone (PTT)/SRT discrepancy as a screen for exaggerated hearing loss

PTT/SRT	Non exaggerators	Exaggerators	
≤10 dB	462	46	508 pass
≥11 dB	13	75	88 fail
Totals	475	121	596

indicate no exaggeration, or alternatively, a discrepancy of more than 10 dB between the speech reception threshold and the puretone average at 500 and 1000 Hz frequency indicated exaggeration. This was put to the test. The results are shown in Table I.

It can be seen that of a total population of 596 patients 79.3% had puretone threshold and SRT responses of 10 dB or closer and of those 91% (46/508) were in fact exaggerators. Of the population where the puretone threshold and speech reception threshold were greater than 10 dB apart, 14% (13/88) were non exaggerators and 85.2% (75/88) were correctly identified as exaggerating. Overall, of those who exaggerated 62% (75/121) were correctly identified.

However, whilst helpful, this still means that 38% of those who exaggerate are missed—a figure which we find unacceptably high.

#### (b) Puretone threshold at 0.5 kHz

The puretone threshold at 500 Hz was examined as a screen for exaggerated hearing loss. Frequency distributions suggested a 40 dB cutoff to attempt to separate patients into those who exaggerated and those who did not.

Table II 40 dB threshold at 0.5 kHz as a screen for exaggerated hearing loss

0.5 kHz threshold	Non exaggerators	Exaggerators	
≤39 dB	268	18	286 pass
≥40 dB	207	103	310 fail
Totals	475	121	596

Looking at this interaction independently (Table II) we found that 52% of the population had a puretone threshold of 40 dB or worse at 500 Hz and a third of these were exaggerators (103/310). However, this cutoff did identify 85% of all exaggerators—better than the SRT test, but still unacceptably low.

#### (c) Combined SRT/PTT and 0.5 kHz criteria

The tests were combined in an attempt to improve the sensitivity. Table III shows the 500 Hz test applied to the 508 patients who 'passed' the SRT/PTT test, a group which included a residuum of 46 so far unidentified exaggerators. If the 500 Hz, 40 dB cutoff is applied to this group along (Table III) a further 37 patients with exaggerated hearing losses are identified, only 9 of the total of 121 (7.4%) are now missed at the price of a false identification of a further 196 patients.

As we combine the tests by failing all those who pass the first but fail the second criterion we correctly identify 112 of a total of 121 exaggerated hearing losses or 93% of the total whilst falsely identifying 209 people (Table IV). Thus of 596 original patients 44.6% (266/596) are correctly identified as giving accurate thresholds, 53.8% (321/596) are identified as giving inaccurate thresholds, and under half of these (112/321) are actually exaggerating. Thus, by means of these two simple tests the number of patients who have to travel to a 'sophisticated' centre may be almost halved at a considerable economic and social saving but the number of patients with missed exaggerated hearing loss is kept down to what is

Table III Application of 40 dB 0.5 kHz threshold to those passing test in Table I

0.5 kHz threshold	Non exaggerators	Exaggerators	
≥40 dB	266	9	275 pass
≤39 dB	196	37	233 fail
Totals	462	46	508

Table IV Application of SRT/PTT and 0.5 Hz criteria to the entire study group

	Non exaggerators	Exaggerators	
Pass both criteria	266	9	275
Fail either criterion	209	112	321
Totals	475	121	596

probably an acceptably low figure, i.e. 7.4% of the exaggerators or 1.5% of the total population.

It has previously been shown by Klockhoff et al (1974) that a hearing threshold of 40 dB or worse may well indicate the presence of other ear disease. Thus the false positive group identified within the narrow context of exaggerated hearing loss, may confirm patients with other ear disease, much of it not previously diagnosed. These patients stand to benefit from further diagnosis at a secondary centre.

To complete the story, the criteria outlined were applied to the total population of patients referred to us with noise induced hearing loss alone. These are patients who did not necessarily have ERA but were sent by the Compensation Board for assessment. The double test criteria were applied to the total computer bank of 1456 patients. Approximately 46% passed both tests and thus potentially do not need sophisticated test facilities, and only 54% need be sent to a major centre—a weak improvement. Further studies are in progress to see if screening in a primary centre will have the same value as it has in a fully equipped diagnostic centre such as ours.

## CONCLUSIONS

Careful screening by means of puretone and speech reception thresholds in NIHL may

limit the need for testing in major centres always with the proviso that if there is a history of ear disease, a discrepancy between the hearing in the two ears, or tester suspicion the patient be referred further.

## ZUSAMMENFASSUNG

Wir untersuchten 596 Patienten, die wegen der Möglichkeit eines laumbedingten Hörverlustes überwiesen worden waren, durch konventionelle Audiometrie und kortikale hervorgerufene Potentiale. Diskrepanzen zwischen beiden Tests identifizierten 121 (20.3%) Fälle, die einen übertriebenen Hörverlust vorspiegelten. Dann prüften wir die Validität einfacher konventioneller Testmethoden jener Art, wie sie in primären diagnostischen Laboren zur Verfügung stehen, indem wir nach den Übertreibern suchten, die vorher identifiziert worden waren. Wenn Fälle mit einer Differenz von mehr als 10 dB zwischen durchschnittlicher Reinton (für 0.5 und 1 kHz) und Sprachrezeptionsschwelle ausgewählt wurden, oder jene, deren initiale Reintonschwelle (bei 0.5 Hz) 40 dB oder mehr betrug, konnten wir 112 (93%) der Übertreiber identifizieren, allerdings auf Kosten von 209 (35%) des Gesamtmaterials falsch positiven Fällen. Dennoch zeigt die Bedeutung dieser Testprozedur, daß 46% der Antragsteller komplett in einem primären Diagnoselabor ausgewertet werden konnten und nur ein geringer Teil der Übertreiber übersehen wurde.

## REFERENCES

- Alberti P W 1970 New tools for old tricks. *Ann Otol Rhinol Laryngol* 79 800
- Alberti P W & LeBlanc J C 1976 An economic evaluation of occupational hearing loss. Paper presented at the 30th Annual Meeting of the Canadian Otolaryngological Society. *J Otolaryngol* (accepted for publication)
- Alberti P W, Morgan P P & LeBlanc J C 1974 Occupational hearing loss—an otological view of a long term study. *Laryngoscope* 84 1822
- Klockhoff I, Drettner H & Svedberg A 1974 Computerized classification of the results of screening audiometry in groups of persons exposed to noise. *Audiology* 13 326

P W Alberti MD  
Suite 405  
600 University Avenue  
Toronto Ont M5G 1X5  
Canada

## PERIPHERAL VASOCONSTRICTION IN THE RAT IN RESPONSE TO SOUND

### II Dependence on Rate of Change of Sound Level

E. Borg

From Department of Physiology II, Karolinska Institutet, Stockholm, Sweden

(Received July 1 1977)

**Abstract.** Vasoconstrictions elicited by sound were studied in the non anaesthetized rat. Arterial pulsations in the tail were recorded by a non invasive technique. On slight heating the animal, the tail vessels became dilated. An 80 dB SPL noise burst caused a decrease in pulse amplitude usually to less than 10% of the pre-stimulus value. It was found that 4 s bursts of 80 dB SPL noise with rise times of 10 or 100 ms were equally efficient in producing vasoconstriction. If the rise time was longer, 1 s the vasoconstriction was significantly smaller. It was pointed out that the feedback control of the stimulus noise provided by the acoustic middle ear reflex would contribute to enhancing rapid variations in sound level and thereby form part of a physiological explanation for the present findings.

Over the years, an increasing interest has been focused on the relation between the auditory system and the autonomous nervous system. Two incentives to this development may be mentioned. Firstly, the possibility of noise being a health hazard, and secondly, the demonstration of the richness of sympathetic innervation of the inner ear (Spoendlin & Lichtensteiger, 1966; Densert 1974). The two basic questions relevant to these problems are largely unanswered: (a) How does sound influence the activity of the autonomous system? and (b) What is the effect of the sympathetic nerves on the ear?

Research in this field has been hampered by the lack of suitable experimental preparations. A recently described animal model has, how-

ever, been found suitable for an approach to the first of the questions mentioned above (Borg, 1977a, b). It was shown that the blood flow in the tail of the non anaesthetized, slightly heated rat was influenced by a sound stimulus to the ear in a reproducible fashion and systematically dependent on sound characteristics.

The vasoconstriction following a sound burst was found to be proportional to sound level from hearing threshold over at least 80 dB (Borg, 1977a) and dependent on duration of the burst corresponding to a time constant for temporal integration in the order of 0.1 s (Borg, 1977b).

The aim of the present study is to continue the analysis of the role of acoustic features for peripheral vasoconstriction. The sensitivity of vasoconstriction to rate of change of sound level has been investigated by using noise bursts with rise times in the range 1 ms to 1 s.

## METHODS

Two series of experiments were performed. Ten adult, male rats participated in each. One of the animals was experimentally naive and animals took part in both sessions. The two sessions were identical except for the choice of rise time values.

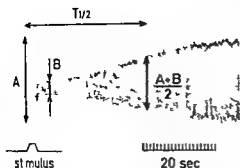


Fig. 1 Recording of arterial pulsations obtained with a non-invasive technique from the tail of the non anaesthetized rat. Decline in pulse amplitude is elicited by a 4 s burst of 80 dB SPL noise with 1 s rise and decay times. The vasoconstriction is quantified by  $T_1$  time from onset of sound until pulse amplitude is halfway normalized.

Since a detailed presentation of the stimulus and recording system has been given earlier (Borg 1977a) the following description of these systems will be brief. During the experimental session the animal rested on a regulated heating pad in an individually adjustable net tube. The blood flow in the tail was assessed in terms of pulsations recorded by a rubber balloon connected to a volume-sensitive transducer (Elema 510C). In room temperature the tail artery was constricted and pulsations were minimal. A moderate heating was necessary to induce vasodilatation. The acoustic-vascular reflex manifested itself as a decrease of pulse amplitude, usually to less than 10% of the prestimulus value. The vasoconstriction was quantified as the duration of the response from the start of the sound until the amplitude of the pulsation had returned halfway to pre-stimulus level ( $T_1$  of Fig. 1). The reasons for using the duration of the response rather than the change of pulse amplitude as a measure of the vasoconstriction have been presented earlier (Borg, 1977a). A Lansing L75 loudspeaker placed 10 cm in front of the rat was used to deliver the sound stimuli.

The stimulus consisted of a burst of a broadband noise with maximum energy between 3 and 15 kHz (see Fig. 2, Borg 1977a). Its sound level was 80 dB SPL (sound pressure level, re

20  $\mu$ Pa). The total duration of the burst was 4 s, its time for linear rise (and decay) being 10, 100, or 1000 ms (in the first series of experiments) and 1, 10 or 100 ms (in the second series of experiments). During each session all of which lasted 4–12 h the differently shaped stimuli were presented several times (3–5) in random order with an interval of at least 15 min.

## RESULTS

A typical recording of a tail artery pulsation during acoustic stimulation is shown in Fig. 1. The acoustic-vascular reflex response to an 80 dB SPL noise burst with a 1 s rise time (and decay time linear) is evident as a decline of pulse amplitude. After a delay of approximately 1 s the pulse amplitude diminishes returning to the normal value after about 1 min. The responses were quantified by  $T_1$  which denotes the time lapse from start of stimulus until the pulse amplitude had returned halfway to pre-stimulus value.

The dependence of the vasoconstriction on rise time in the first series of experiments is illustrated in Fig. 2. The thin continuous lines

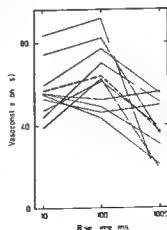


Fig. 2 Duration of vasoconstriction as a function of rise time (10, 100 and 1000 ms) of a 4 s burst of 80 dB SPL noise. Each thin continuous line represents one animal in which up to 5 determinations at each rise time were obtained. Heavy broken line shows mean values.



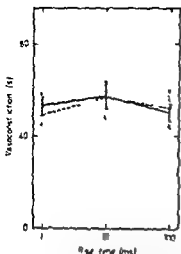


Fig. 1. Duration of vasoconstriction as a function of rise time (1, 10, and 100 ms) of a 4 s burst of 80 dB SPL. Average and standard error of the mean — Median and semi-interquartile range.

represent average responses to 4 s noise bursts with rise times of 10, 100, and 1000 ms in each of the ten animals. The heavy broken line shows mean values. It is seen that bursts with rise times of 10 and 100 ms give about equal responses, whereas those having a 1 s rise time are usually followed by shorter vasoconstrictions. The difference between 100 and 1000 ms responses is statistically significant (Student's *t*-test at 1% level). The difference between responses for rise times of 10 ms and 1000 ms is significant at the 5% level (Student's *t*-test). One may also get the impression that bursts with 10 ms rise times are less efficient in producing vasoconstriction than are those with 100 ms rise time.

In order to analyse the short rise time range (1–100 ms) further, the second series of experiments was performed. Fig. 3 shows mean values and standard error together with median and semi-interquartile ranges from experiments in 10 rats. It appears that bursts with rise times from 1 to 100 ms are equally effective in eliciting vasoconstriction.

In conclusion, sound bursts which are slowly changing in level elicit smaller vasoconstrictions than do bursts with rapidly changing intensity.

## DISCUSSION

The results show that a 4 s noise burst (80 SPL) elicits a smaller vasoconstriction when the rise- and decay times are long, 1 s than when they are short, 1, 10, or 100 ms. The bursts with faster rise are, on the other hand, equally efficient. This difference is not a consequence of the shorter effective duration of the burst with 1 s rise and decay time (plotted at 80 dB SPL, 2 s). It has been shown previously, that noise bursts with 1 s and 4 s give almost identical vasoconstrictions as long as they have equal rise times (Borg 1977b).

Evidence exists indicating that rise time is important for the ability of sound to influence sleep. LeVere et al. (1976) showed that sound with rapid onset (relaxation controlled) was effective in causing disturbance of sleep than slowly rising (7.5 s rise time) sound. On the other hand, according to Nixon et al., noises with very slow onset (15 s) appear more annoying than suddenly occurring. This observation was explained by the assumption that the increasing level is perceived as being produced by an approaching object. An adequate comparison between annoyance, change of sleep stage, and vasoconstriction can be made only when observations on identical stimuli are available. This is unfortunately not the case.

The low sensitivity of the acoustic-vasoconstrictor reflex to slowly rising stimuli may be caused by negative feedback control in the lower auditory system. A negative feedback effect on the acoustic signal or on the neural message tends to enhance rapid variations in amplitude compared with slow variations. The following remarks will be limited to the best known of the many feedback systems in the auditory pathway, the acoustic middle ear muscle reflex. The intensity and frequency of sound used in the present experiments are large within the ranges controlled by the middle ear muscles in animals (Borg 1972). If the time is short, a significant part of the acoustic energy will escape the reflex attenuation provided by the muscles due to their relax-

low dynamic properties (Borg, 1976). When the middle ear muscles are excited by a tone with 2-10 ms rise time, full attenuation is provided after 200 ms in humans, and after 50 ms in rabbits. In rats, the figure is not known but most probably full attenuation is reached after 50 ms or somewhat less. When onset of sound is slow, the time to full effect of the feedback control is somewhat longer. No measurements with 100 ms and 1 ms rise times are, however, available. Thus, the attenuation of the stimulus provided by the middle ear muscles may be suggested to contribute to decrease the efficiency of bursts with 1 ms onset in comparison with bursts with shorter rise time. A more complete account of the underlying mechanisms can not be presented until the neuronal pathway of this acoustic-vascular reflex is established.

In summary, the present experiments show that the acoustic-vascular reflex in the tail of the rat is rate sensitive properties.

### ZUSAMMENFASSUNG

An narkotisierten Ratten wurden die durch Tonpulse ausgelösten Gefäßkontraktionen untersucht. Dies wurde durch Aufzeichnung der arteriellen Pulsationen im Schwanz mittels einer nichtinvasiven Methode durchgeführt. Während der Erwärmung des Tieres wurden die Blutgefäße im Schwanz erweitert. Es konnte gezeigt werden, daß Geräuschstöße von 100 dB SPL eine Abnahme der Pulsamplitude bewirken und zwar im allgemeinen auf weniger als 50% des Wertes vor der Stimulation. Weiter

wurde festgestellt, daß Geräuschstöße von 4 s Dauer und 80 dB SPL bei Steigzeiten von sowohl 1 wie 10 oder 100 ms praktisch die gleichen Gefäßkontraktionen hervorrufen. War jedoch die Steigzeit länger (1 s) so war die folgende Gefäßkontraktion signifikant geringer. Eine physiologische Erklärung dieses Verhaltens konnte dann gesehen werden, daß die Rückkopplungskontrolle durch den akustischen Mittelohrreflex auf den Geräuschstimulus dazu beiträgt, steile Änderungen im Geräuschpegel zu vergrößern.

### REFERENCES

- Borg E 1972 Acoustic middle ear reflexes. A sensory control system. *Acta Otolaryngol* (Stockh) Suppl 304.
- 1976 Dynamic characteristics of the intra-aural muscle reflex. *Acoustic impedance and admittance—the measurement of middle ear function* (eds A S Feldman & L A Wilber). Williams & Wilkins.
- 1977a Tail artery response to sound in the unanesthetized rat. *Acta Physiol Scand* 100: 129.
- 1977b Peripheral vasoconstriction in the rat in response to sound. I. Dependence on stimulus duration. *Acta Otolaryngol* (Stockh) in press.
- Densert O 1974 Adrenergic innervation in the rabbit cochlea. *Acta Otolaryngol* (Stockh) 71: 345.
- LeVere T E, Davis N, Mills J, Berger E H & Reiter W F 1976 Arousal from sleep: effects of rise time of auditory stimuli. *Physiol Psychol* 4: 213.
- Nixon C W, Von Gierke H E & Ravinger G 1969 Comparative annoyance of approaching versus receding sound sources. *J Acoust Soc Am* 45: 330.
- Spoendlin H & Lichtensteiger W 1966 The adrenergic innervation of the labyrinth. *Acta Otolaryngol* (Stockh) 61: 423.
- E Borg MD  
Dept of Physiology II  
Karolinska Institutet  
S 10401 Stockholm  
Sweden

## REFLEX INHIBITION AUDIOMETRY

### *A New Objective Technique*

R. R. Marsh, II, S. Hoffman and C. L. Stitt

*From the Department of Psychology, Bryn Mawr College, Bryn Mawr, PA, USA*

(Received June 21, 1977)

**Abstract** The reflexive eyeblink elicited by a tactile stimulus is inhibited if an auditory stimulus precedes the eliciting stimulus by 100 msec. With adult subjects the threshold for this effect was found to be sufficiently low to suggest that reflex inhibition may be useful in objective audiometry. A preliminary investigation with infants and children showed that the inhibitory process is present, though variable, in children as young as 6 weeks old.

One of the more difficult problems facing the audiologist is the evaluation of the nonverbal child. Although several objective techniques are available, each has some limitation, such as cost in equipment or time, or unsuitability for very young or multiply handicapped patients. We report here a new objective technique which shows promise of overcoming some of these barriers. In this test one assesses hearing acuity by determining whether the reflexive eyeblink (elicited by tactile stimulation of the face or cornea) is inhibited by an acoustic stimulus which precedes the non-acoustic reflex eliciting stimulus by a fraction of a second. This technique, which we have named reflex inhibition audiometry, requires no active cooperation on the part of the patient nor does it involve learning or conditioning. These two characteristics in particular would recommend its use with the difficult-to-test child.

Reflex inhibition audiometry had its origin in a series of investigations of the acoustic startle reflex in the rat. This reflex, as measured with a stabilimeter, was found to be in-

hibited if a single relatively weak pulse of noise preceded the more intense startle stimulus by 20-500 msec (Hoffman & Searle, 1965). Further investigation showed this inhibition to be robust, persisting after hours of presentation of the inhibitory stimulus (Marsh et al., 1969), and to be independent of associative or conditioning processes; inhibition may be found on the first trial on which the startle stimulus is preceded by a pulse of noise (Krauter et al., 1973).

The inhibitory process also proved to be rather sensitive, inhibition appearing in the rat when a 35-dB SPL noise burst precedes the intense startle eliciting stimulus by 150 msec (Hoffman & Wible, 1970). This sensitivity has led to speculation that a comparable paradigm might have an application in audiometry: a modification of the reflex by an appropriate prestimulus would verify the patient's acuity for tones of that frequency and intensity. Investigation of this possibility was at first frustrated for two reasons. Inhibition was at the time believed to be specific to the acoustic startle reflex, and the use of intense startle stimuli with humans would have been inappropriate. The second obstacle was the measurement of the startle reaction. This response is easily measured with a stabilimeter when small animals are used, but more sophisticated

The work was supported by Grant MH-20444 from the National Institute of Mental Health, directed by Howard S. Hoffman.

Jures such as electromyography, would  
 and with humans

Recent research has shown that reflex in-  
 hibition might be a feasible audiometric proce-

Overall First came the discovery that the  
 startle-eliciting stimulus and the inhibitory  
 stimulus need not be of the same sensory

type The acoustic startle reflex of the rat  
 is inhibited by a visual prestimulus, and the

visually elicited startle is inhibited by  
 auditory prestimulation (Buckland et al., 1969,

et al. 1976) These studies expanded the  
 range of available eliciting stimuli by demon-

strating that inhibition by auditory prestimula-  
 tion is simply from an interaction

with a subsequent auditory eliciting stimulus  
 More encouragement came from the observa-

tion that prestimulation effects can be found in  
 the human when only the eyeblink is measured

instead of the skeletal startle response (Gra-  
 ham 1975) furthermore, inhibition of the re-

flexive eyeblink by acoustic stimulation has  
 been demonstrated when the eyeblink itself

was elicited by a gentle puff of air (Krauter et  
 al. 1973) These were particularly important

observations because the eyeblink can be safely  
 and objectively measured, as psychologists

have done for years in the course of classical  
 conditioning studies, and because tactile

stimulation such as that used by Krauter et al.  
 would certainly be more appropriate than in-

terrupting auditory reflex eliciting stimuli for  
 evaluating the hearing impaired patient

These observations have stimulated addi-  
 tional research with humans which laid the

groundwork for the research reported here  
 Those studies confirmed the sensitivity of

the inhibitory process—inhibition was demon-  
 strated with a 30-dB SL tone burst preceding

the tactile eyeblink eliciting stimulus—and  
 demonstrated that the amount of inhibition

found with a tone burst of fixed intensity was  
 independent of the intensity of the eliciting

stimulus i.e., the antecedent tone reduced the  
 response by a constant amount which was in-

dependent of the baseline response amplitude  
 (Marsh 1976) These data showed that the

procedure could be readily administered at  
 least with adults, and that great attention to  
 the intensity of the reflex-eliciting stimulus—a  
 puff of air striking the face lateral to the eye—is  
 unnecessary In spite of these encouraging  
 results, reflex inhibition still did not appear  
 to be entirely satisfactory The inhibitory ef-  
 fect of weak tones, while statistically demon-  
 strable, was not great, and certain subjects  
 failed to show inhibition at all

At this point reflex inhibition by monotic  
 prestimulation was attempted in contrast to all  
 previous research where inhibitory stimuli  
 were presented binaurally in a sound field or  
 by earphones Contrary to expectation  
 monotic prestimuli inhibited the eyeblink re-  
 flex to a much greater extent than did binaural  
 stimuli (Marsh et al. 1976) Even those sub-  
 jects who had failed to show any inhibition  
 before, inhibited quite reliably when retested  
 with monotic inhibitory stimuli The same  
 study examined the symmetry of reflex in-  
 hibition by testing all left-right combinations of  
 auditory prestimulus-tactile eliciting stimulus  
 and response transducer No interaction was  
 found between ear under test and either of the  
 other two variables implying that the reflex-  
 eliciting stimulus delivery device and response  
 transducer may each be arbitrarily applied to  
 one side of the face or the other without re-  
 gard to which ear is to be tested

## EXPERIMENT I

This study served to integrate the results of  
 the above cited experiments and to provide an  
 indication of the potential performance of re-  
 flex inhibition audiometry in clearing com-  
 parable populations of suspicion of hearing  
 loss So that this study would better address it-  
 self to clinical issues, tone intensities were set  
 with reference to ANSI norms rather than  
 being specified as sensation levels also a  
 range of audiologically significant frequencies  
 were examined, rather than the single 1 kHz  
 tone employed in other human research in this  
 laboratory

## Method

**Subjects** Twelve graduate and undergraduate students at Bryn Mawr College served in this experiment

**Apparatus** The apparatus typically used in experimental investigations employing the eyeblink, as in classical conditioning research, does not lend itself well to the ultimate goal of clinical applications where cost, portability, and patients' comfort are important considerations. For this reason equipment which meets these requirements was designed for the research reported here.

The device for generating the eyeblink-eliciting airpuff was an air suspension acoustic speaker modified to produce a highly reliable airpuff. The diaphragm of the speaker was coated with silicone rubber and the speaker was enclosed in such a way that displacement of the diaphragm forced air through a tube leading to the subject. The airpuff was generated by charging a bank of capacitors (400  $\mu$ F) to 200 V, then discharging them through the modified speaker by means of a silicon controlled rectifier with a 1-henry inductance in series to filter out audio frequency components from the current surge. An 8 ohm attenuator was constructed to permit adjustment of the airpuff intensity without affecting the load experienced by the solid state capacitor-discharge circuit.

The eyeblink was measured with a modified d'Arsonval meter fastened to the end of the air delivery tube. The pointer on the meter was extended with a length of polyethylene tubing which was taped to the eyelid. This unit translated movements of the eyelid into rotation of the meter coil in a magnetic field and generated a voltage proportional to the velocity of the motion. This voltage was amplified, rectified, and measured by a digital voltmeter with storage capability.

To simplify preparation of the subject, the air delivery tube response transducer assembly was attached directly to the earphone headset used for the delivery of acoustic signals. A specially designed clamp permitted

rapid adjustment of this assembly so that the airpuff struck the face just lateral to the eye.

The tones which served as antecedent stimuli were generated by controlling the output of a Hewlett-Packard Model 200A V audio oscillator with a Grason-Stadler electronic switch (Model 829 D). A General Radio decade attenuator (Type 1450-TB) permitted adjustment of tones with reference to A<sub>1</sub> (1969) norms, and a Daven A-1023-G attenuator permitted independent adjustment of stimuli in 2-dB steps. Auditory stimuli were delivered through TDH 39 earphones fitted with MX-41/AR cushions. The earphones test stimuli were calibrated with a C Radio precision sound level meter (V 1561-A), fitted with a P 7 microphone (ANSI Type 1 coupler, using ANSI 11 norms).

Grason-Stadler Model 471-1 interval controlled the duration of prestimuli as well as their lead time, measured as the interval between arrival of the leading edge of the stimulus and the leading edge of the airpuff and taking into account delays involved in transmission of the airpuff through the tube from its generator to the subject. A solid state and relay circuitry operated the airpuff generator under the control of the timers.

All studies were conducted in an 11 double wall sound treated room having ambient noise level below 25 dBA. This chamber was suitably furnished and lighted, equipped with a rear projection screen so 35 mm slides could be projected from the control room. A closed-circuit television camera and an intercom permitted continuous monitoring of the subject.

**Procedure** After each subject had appraised of the nature of the research and agreed to participate, he or she was placed in the chamber and fitted with the headworn earphones, airpuff delivery tube, and response transducer. The subject's thresholds for the tones employed in this study were determined by the method of Carhart & Jer-

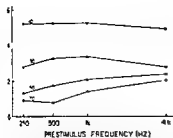


Fig. 1. Response amplitudes in airpuff stimulation with stimuli of several audiological significant intensities (indicated as the parameter in dB HL) and frequencies (indicated as the parameter in Hz). The -10 dB HL prestimulus provide a basis for assessing the inhibitory effects of the other prestimuli.

959) this clinical technique being modified so that test tones were varied in increments of 2 dB rather than 5 dB.

During the experimental session itself the desired combinations of antecedent auditory stimulus and blink eliciting airpuff were presented at 30-sec intervals. To combat boredom 35 mm color slides of paintings or nature objects (wild animals, scenic views, etc.) were shown to the subject on the rear projection screen in the chamber. Except for a brief interval between trials when slides were changed, they were projected continuously. In experimental sessions, including briefing and preparation of the subject, required no more than 30 minutes to complete.

Each subject was evaluated for one ear only: half for the right and half for the left. Prestimuli having frequencies of 250, 500, 1000 and 4000 Hz were presented, each at intensities of -10, 30, 50, and 70 dB HL, each frequency-intensity combination being presented eight times over the course of two sessions. Every prestimulus had a rise time of 10 msec, a 200-msec duration, and an onset preceding the airpuff by 100 msec. The rise time employed here, although less than that specified for clinical audiometers, had been found to be somewhat more effective than longer rise times in inhibiting the eyeblink reflex (Marsh, 1976).

### Results and Discussion

Examination of the averaged responses (Figure 1) shows prestimuli of 30 dB HL to strongly in-

hibit the eyeblink reflex, relative to the sub-threshold -10-dB condition, for all frequencies tested. A two way repeated measures analysis of variance confirmed a significant effect of prestimulus intensity,  $F(3, 33)=115.58$ ,  $p<0.01$ . Although no effect of frequency was demonstrated,  $F(3, 33)=1.96$  n.s., a significant frequency-intensity interaction was found,  $F(9, 99)=2.76$ ,  $p<0.01$ .

Scores of individual subjects are naturally of more interest to the clinician than are group averages. Each subject had smaller average responses at 30 dB HL than at -10 dB for every frequency except for a single subject at a single frequency, she did show substantial inhibition by the 50-dB prestimulus at that frequency. It must be admitted that inhibition by 30-dB prestimuli was not so strong for every subject as to be clearly discriminated from random variation, but it should also be remembered that this study only employed eight observations per stimulus condition. Moreover, if a one volt response decrement is arbitrarily taken to be evidence of inhibition, the data remain encouraging. Seven subjects met this criterion for all frequencies at 30 dB and all did for every frequency at 50 dB except for two subjects who failed to reach this criterion at 50 dB but did so at 70 dB for a single frequency, even they met the requirement at 50 dB for three frequencies.

### EXPERIMENT 2

Although research with adults yielded encouraging evidence in support of the potential of reflex inhibition audiometry, it provided no assurance that the technique would be effective with infants and young children in the age range where objective techniques are most needed. Because of this consideration the reflex inhibition procedure was applied to a number of children ranging from 8 weeks to 5 years of age. These studies were less rigorous in design and execution than the adult research, having the primary objective of providing assurance that reflex inhibition could be found throughout this age range.

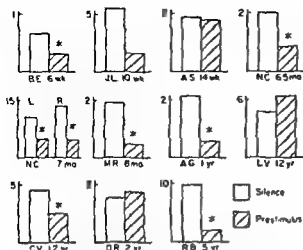


Fig. 2 Individual reflex amplitudes in infants and children measured in silence and with a 1 kHz 70-dB HL pre-stimulus. An asterisk indicates significant response inhibition by the prestimulus ( $p < 0.05$ ).

## Method

**Subjects** Eleven babies and children participated in this study. All except one, the daughter of a college employee, were recruited for this work by their family physician. None were suspected of any hearing impairment.

**Apparatus** It proved desirable to modify the equipment used with adults to make it more suitable for infants. It was found advantageous to attach the response transducer directly to the infant's forehead. The face of the meter housing was secured to the forehead above the eye with adhesive material of the kind used to attach ostomy appliances. A strut constructed of polyethylene tubing linked the meter's needle to the eyelid, where it was attached with a small strip of Micropore tape. This device which weighs less than 14 gm and is quite small (approx.  $2 \times 4 \times 4$  cm) has proved to be easy to apply and comfortable for the infant. The eyelid attachment linkage is quite safe and well tolerated, being of soft material with smooth edges.

The airpuff delivery tube was connected to a hand-held unit which was placed about 3 cm in front of the infant's eye during each trial. This handpiece was constructed from a soft plastic pacifier and presented no hard surfaces which might injure the baby. The shape of the

device, with its soft eyeshield (the nipple of the pacifier having been removed) further protected the infant as it presented a large flat surface which could not enter the eye. The acoustic test stimuli were delivered to the baby via a single earphone (TDH39 fitted with an MX41 AR cushion) that was held against the baby's ear at the start of each trial. A pushbutton switch on the handpiece of the air delivery system permitted the tester to initiate a trial when the handpiece and earphone were correctly positioned.

**Procedure** During a test session the subject sat on its parent's lap. One experimenter in the control room selected the stimulus configuration to be employed and the other experimenter, who was with the infant, was notified by means of a signal light when a trial was to begin. He then positioned the earphone and airpuff delivery handpiece and initiated the trial. This experimenter had no information as to the stimulus employed on a particular trial or the amplitude of the infant's eyeblink.

Ten airpuffs were presented in silence interspersed with ten airpuffs preceded by a 70 dB HL, 1-kHz tone, usually having a 100 msec lead time, although 200 msec was used with several subjects. As in Experiment 1, the tone had a 10 msec rise time and continued for 100 msec after the airpuff. All subjects were tested with one ear only, except for NC who returned for a second session in which both ears were tested. The procedure proved to be comfortable for the babies and children who gave no evidence of annoyance during the test.

## Results and Discussion

The data for each subject are shown in Fig. 2. An asterisk indicates that the responses in the prestimulus and stimulus conditions were significantly different according to a two-tailed  $t$ -test on that subject's data,  $p < 0.05$ . The successful demonstrations of reflex inhibition cover the entire age range. As seen in Fig. 2 several subjects did not exhibit reliable differences between conditions. It must be re-

recognized however, that the present work represented only an initial effort. It seems possible that in future work such failures could be prevented by a choice of stimulus parameters more appropriate to this young population.

## GENERAL DISCUSSION

Although a declaration of the efficacy of reflex inhibition audiometry would be premature, this technique does show great promise. The procedures involved have proven themselves simple to administer and safe and comfortable for the subject or patient. The investigation with infants was especially encouraging in demonstrating the ease with which even such young subjects can be tested and confirming their lack of irritation by the apparatus or stimuli. Reflex inhibition commends itself also in requiring relatively inexpensive equipment compared to that required for certain other objective procedures and, if the adult research is any indication, sufficient sensitivity to clear many patients of suspicion of even a moderate hearing loss.

Caution dictates of course that no diagnosis of hearing loss should be based on reflex inhibition audiometry until thorough clinical validation of the technique has been completed. The preliminary work with infants certainly supports such a caveat. Although some subjects of every age showed inhibition, others did not and additional research is needed to identify the factors responsible for this variability.

Relatively little clinical evaluation of the procedure would be required, however, to permit its use as a screening test with infants since the presence of inhibition with a given test stimulus is strong evidence that the tone is above the patient's threshold. Used in this way reflex inhibition audiometry would seem to have great potential in clearing young patients so that only those whose responses were inconclusive would be subject to more time consuming and expensive test procedures. Reflex inhibition audiometry might be valuable

too as a confirmation of audiograms determined by other means, a rapid test at one or two frequencies would lend confidence to the previous determination if good agreement is found.

## ACKNOWLEDGEMENT

These studies were conducted as a part of the first author's doctoral dissertation at Bryn Mawr College.

## ZUSAMMENFASSUNG

Das reflexive Blinken des Auges das bei den Tasterstimulus hervorgerufen ist wird gehemmt wenn ein akustischer Stimulant den auslösenden Stimulant um 0.1 Sekunde vorangeht. Bei erwachsenen Versuchspersonen ist die Reizschwelle für diesen Effekt sehr gering und weist darauf hin daß dieser hemmende Reflex für die Audiometrie verwendet kann. Eine einleitende Untersuchung mit 6wöchigen und älteren Säuglingen und Kindern weist ebenfalls auf diese hemmenden Reflexe hin.

## REFERENCES

- Buckland G, Buckland J, Jamieson C & Ison J 1969 Inhibition of startle response to acoustic stimulation produced by visual presumption. *J Comp Physiol Psychol* 67: 493.
- Carhart R & Jerger J 1959 Preferred method for clinical determination of pure tone thresholds. *J Speech Hear Disord* 24: 310.
- Graham F 1975 The more or less startling effects of weak prestimulation. *Psychophysiol* 17: 238.
- Hoffman H & Searle J 1965 Acoustic variables in the modification of startle reaction in the rat. *J Comp Physiol Psychol* 60: 53.
- Hoffman H & Wible B 1970 Role of weak signals in acoustic startle. *J Acoust Soc Am* 47: 489.
- Krauter E, Leonard D & Ison J 1973 Inhibition of human eyeblink by brief acoustic stimulus. *J Comp Physiol Psychol* 84: 246.
- Marsh R 1976 Modification of the human eyeblink reflex by antecedent stimulation: implications for objective audiometry. Dissertation Bryn Mawr College.
- Marsh R, Hoffman H & Stein N 1969 Persistence of background acoustic stimulation in controlling startle. *J Comp Physiol Psychol* 68: 280.
- Marsh R, Hoffman H & Sott C 1976 Eyeblink inhibition by monaural and binaural stimulation. One ear is better than two. *Science* 197: 390.
- Sott C, Hoffman H, Marsh R & Schwartz G 1976 Modification of the pigeon's visual startle reaction by the sensory environment. *J Comp Physiol Psychol* 90: 601.

R R Marsh Ph D  
Dept of Psychology  
Bryn Mawr College  
Bryn Mawr PA 19010 USA



## EXTRA INTERNAL HAIR CELLS A Scanning Electron Microscopic Study

I Kawabata and Y Nomura

*From the Department of Otolaryngology, University of Tokyo, Tokyo, Japan*

(Received June 21, 1977)

**Abstract** The extra internal hair cell (EIHC) of the human cochlea was observed by means of a scanning electron microscope. The EIHC was found not infrequently in all turns of the human cochlea. It was located medial to the IHC row. The inner pillar cells showed an abnormal structure. The anatomical relationships between the displaced IHC and EIHC and the inner pillar cell were classified into five types. The origin of these anomalies is discussed from an embryological viewpoint.

It has been said that the internal hair cells show less abnormality than the external hair cells in regard to arrangements of cells as well as morphology. However, extra internal hair cells (EIHC) are not infrequently observed when examining the surface of the organ of Corti under the scanning electron microscope (SEM). As early as 1881, Retzius already reported on this and named this phenomenon "überschüssige innere Haarzellen". Kolmer described a similar finding in a light microscopic study (Fig. 1).

While surveying the surface of the human organ of Corti, we have found that abnormal shape and arrangement of the supporting cells such as the inner pillar cells and border cells are observed in the organ of Corti adjacent to EIHC. The purpose of this paper is to describe the morphology of EIHC and to classify cell arrangements of the extra internal hair cells and the supporting cells and to review the available literature.

### MATERIAL AND METHOD

Temporal bones from aged people of over 65 years old who died of pneumonia and heart diseases were used in this study. The bones were removed at autopsy about one hour post mortem and fixed in 10% formalin solution. The membranous labyrinth was removed from the temporal bone and, was then fixed in 2.5% glutaraldehyde solution (0.2 M phosphate buffer) at pH 7.4 for 12-24 hours. Subsequently the materials were fixed with 2% tannic acid solution and 2% osmic acid solution according to Murakami's method. Specimens were dried using the critical point drying method after dehydration through graded alcohol and amyl acetate. They were then coated with gold palladium in a vacuum evaporator (ion sputtering). The specimens were observed under a scanning electron microscope (JSM S type).

### RESULTS

The internal hair cells (IHC) form a single row in the organ of Corti. In the aged, the external hair cells showed a loss of stereocilia, whereas this loss was much less marked in the IHC. Instead, extra cells which lie outside of the usual single row were found in the IHC. The EIHC usually exists medial (toward the modiolus) to the normal position of the IHC row—never on the outside of it. Under low power viewing

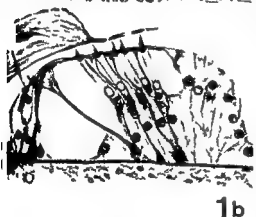
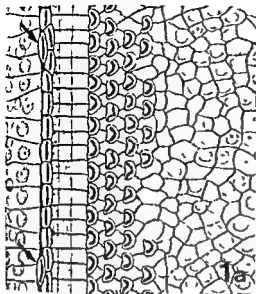


Fig 1 (a) An extra internal hair cell. In 1881 Retzius described a similar duplication of an internal hair cell and termed it "überschüssige innere Haarzellen" (arrow). In 1917 Kolmer described a similar finding in a tangential section of the spiral organ with a light microscopy (low  $\times$ ).



Fig 2 A low magnification SEM picture of the surface of spiral organ. An extra internal hair cell (arrow) exists medial (toward the modiolus) to the normal position of the internal hair cell row (IHC). The internal hair cell row forms a convex curve laterally (toward the external hair cell (OHC))  $\times 800$ .

Fig 3 indicates the normal arrangement of the IHC and supporting cells. The cuticular plate of the IHC is elliptical and it protrudes a bit further than the free surface of the surrounding supporting cells. The long axis of the elliptical cuticular plate lies parallel with the row of IHC.

Lateral to the IHC there is a head of the inner pillar cells. Its head shows a rectangular shape, the long axis of which is perpendicular to the row of IHC. It is tightly in contact with the cuticular plate of the IHC.

Medial to the IHC there is a border cell which has microvilli on its surface. The border cell extends partly into the intercellular space between IHCs. Therefore the border cell and the head of the inner pillar cell contact each other.

As the terminal web was poorly developed in the border cell, its cell margin was not clear. The shape and size of the border cell is, therefore, obscure. Medial to the border cells is the inner sulcus cell, the free surface of which has no microvilli.

1b- IHC row formed a convex curve laterally at the point where the extra cell existed (Fig 2). There was no predominance of the localization of the EIHC in any part of the cochlea. They were found in the upper, middle and the basal turns of the human cochlea.

Under a high power view, no particular structure was found in the cuticular plate and stereocilia in these EIHCs. However, the supporting cells around the EIHC showed unusual cell arrangement.





Fig. 9. Extra internal hair cells are located closely (a) or away (b) from the IHC. (a)  $\times 800$ , (b)  $\times 7400$ .

The anatomical relation of the EIHCs and supporting cells can be classified as follows.

**Type A (Figs 4 and 10A).** This is a dislocation of IHC rather than EIHC. A displaced IHC is seemingly pushed medially from the normal IHC row by a head of the inner pillar cell.

**Type B (Figs 5 and 10B).** There is a gap in the IHC row. However, the head of the inner pillar cell is in normal position. This IHC is medial to the IHC row. The border cells with numerous microvilli surround the IHC. In other words, this type has no extra IHC but rather a dislocation of an IHC. The original area is replaced by the border cell.

**Type C (Figs 6 and 10C).** The IHCs lie side by side tightly. An EIHC is medial to and close to the IHC row.

**Type D (Figs 7 and 10D).** Like Type C, there is no deficit of an IHC and in the IHC row. However, an EIHC is present medial to and away from the IHC row. The border cell with numerous microvilli is between the EIHC and the IHC row.

**Type E (Figs 8 and 10E).** This type shows a mixture of types A and D. An EIHC is located medial to and close to the IHC row. The head of the inner pillar cell passing through the IHC row is in contact with the EIHC's cuticular plate. The head of the inner pillar cell is not rectangular but shows a curved shape.

The EIHCs are observed at times located side by side or closely (Figs 9a, b). A scheme of the above classification is shown in Fig. 10.

Fig. 10. High magnification SEM pictures of normal and extra internal hair cells.

Fig. 10A shows the normal arrangement of an internal hair cell and the supporting cells. An internal hair cell (IHC) is located between the head of internal pillar cells (IPC) and border cells (BC).  $\times 2400$ .

Fig. 10B shows an extra internal hair cell corresponding to the IHC in Fig. 10A. An extra internal cell (a) moves toward the border cells with the head of the internal pillar cells.  $\times 2400$ .

Fig. 10C shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10D shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10E shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10F shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10G shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10H shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10I shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10J shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10K shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10L shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10M shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

Fig. 10N shows an extra internal hair cell (a) located in the IHC row.  $\times 2400$ .

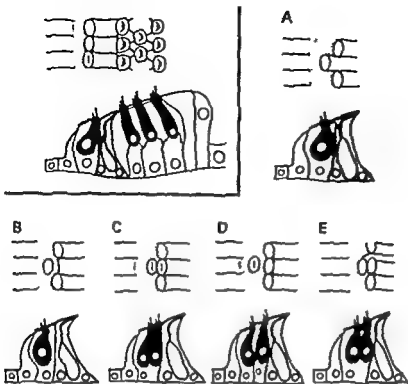


Fig 10 A schematic drawing of the classification of the extra internal hair cells

## DISCUSSION

Little has been mentioned about the extra internal hair cell (EIHC) since the first description of Retzius and later Kolmer. Recently Soudyn described briefly the EIHC of the guinea pig using the SEM. The paucity of the literature is mainly attributable to a low incidence of EIHC (Kolmer, 1927, Engstrom et al, 1970).

The EIHC exists in the organ of Corti of man (Retzius, 1881; Kolmer, 1927), rabbit (Retzius, 1881) and guinea pig (Soudyn, 1976). It is also found in rat, mouse and dog. Therefore, it is reasonable to assume that the EIHC is present in the organ of Corti of many species and not specific to any one species. It is our impression that there are more EIHCs in the human organ of Corti than in other animals.

As to the location of the EIHCs, Soudyn reported that they are found more in the upper turn of the guinea pig. Kolmer's specimen was also from the upper turn of the cochlea. In the present investigation, the EIHCs were found

more in the apical turn of the human organ of Corti, though they were also widely distributed in the middle and basal turns.

The cytoarchitecture of the IHC and supporting cells was investigated in detail by transmission electron microscopy (Iurato, 1967; Kimura et al, 1965; Kimura, 1975). According to Kimura (1975), the supporting cell of the IHC are the inner pillar cell, the inner phalangeal cell, the border cell and the inner sulcus cell. The inner phalangeal cell is located between the inner pillar cell and the IHC. It is a small cell, irregularly shaped and having many microvilli on the free surface. The border cell is located between the IHC and the inner sulcus cell.

On observing the fine surface of the human organ of Corti by means of SEM, the inner pillar cells and the inner sulcus cells were easily distinguishable, whereas the inner phalangeal cells and the border cells were hardly distinguishable. The microvilli observed in the narrow space between the IHCs is possibly on the free surface of the inner phalangeal cell.

• found it difficult to distinguish the inner lateral cell from the border cell even when there was a relatively wide space between the IHCs (Fig. 5) the border cell is defined as having numerous microvilli on its free surface.

In this paper we propose to classify the array of EIHC and supporting cells into five types. The head of the inner pillar cell displayed an abnormal shape, whereas the border cell and the inner sulcus cell do not show any abnormality.

Considering combinations of the presence or absence of abnormality of the IHC row and head of the inner pillar cell, the above mentioned five types can be grouped as follows:

**Group I** There is no gap in the IHC row. As the EIHCs lie medial (toward the modiolus) to the normal IHC row, the inner pillar cells show no abnormality.

**Group II** There is a gap in the IHC row. No abnormality in the head of the inner pillar cell.

**Group III** There is a gap in the IHC row. The pillar head extends toward the modiolus.

Retzius observed the EIHC in a newborn infant. Soudyn (1976) thought the EIHC to be a normal variation. Bredberg (1968) found the EIHC in a 6-month old foetus. The cells in the organ of Corti are quite regularly arranged in the foetus. After birth, hair cells show an irregular arrangement. However, we are of the opinion that the EIHC observed in aged persons in the present study are formed during the development of the inner ear. It is still a matter of dispute as to which turn of the cochlea develops first (Pujol & Marty, 1970). However, studies of Kolmer (1927), Kollmann (1898) and von Nothmann (1894) have been generally accepted regarding the development of the organ of Corti. According to Kolmer, differentiation of the organ of Corti begins as early as the 3rd fetal month in the epithelial mound of the cochlear duct. The organ of Corti develops fully from the 3rd to 4th foetal month. Thickened epithelium becomes the neuroepithelium. Three rows of the external hair cells are differentiated from a lateral part of

the neuroepithelium whereas one row of IHC results from a medial part.

In the 7th month the inner sulcus is formed at the same time, and a space is built between the IHC and the external hair cells. This develops into the tunnel of Corti which is formed by the inner and outer pillar cells.

The hair cell is differentiated and completed from the neuroepithelium prior to the formation of the pillar cell. Considering the various arrangements of EIHC from the organogenesis of the organ of Corti, anomaly of cell arrangement occurs in the early stage of development in Group I. The EIHC with anomaly of head of the inner pillar cell observed in Group III seems to arise in a relatively late stage of development.

The function of the EIHC is unknown. Since the IHC is innervated much more than the external hair cell in the human organ of Corti (Nomura, 1976) it is important to study whether or not the EIHC has a pattern of innervation similar to that of the IHC.

## ZUSAMMENFASSUNG

Die überschüssigen inneren Haarzellen der Schnecken von Menschen wurden mit dem Raster Elektronenmikroskop betrachtet. Diese Zellen befinden sich auf der ganzen Windung der Schnecke und zwar innerhalb der inneren Haarzellen Reihe. Auch an den nebenliegenden inneren Pfeilerzellen wurden Anomalien beobachtet. Die anatomischen Beziehungen zwischen den verschobenen inneren Haarzellen, überschüssigen inneren Haarzellen und inneren Pfeilerzellen wurden in 5 Typen geteilt und die Bildung dieser Anomalien wurde aus den genetischen Ansichten besprochen.

## REFERENCES

- Bredberg G 1968 Cellular pattern and nerve supply of the human organ of Corti. *Acta Otolaryngol* (Stockh) Suppl 236.
- Engstrom H, Ades H W & Bredberg G 1970 Normal structure of the organ of Corti and the effect of noise induced cochlear damage. In: A Ciba Foundation Symposium: *Sensorineural Hearing Loss* (ed G E W Wolstenholme & J Knight). J & A Churchill, London.
- Iurato S 1967 *Submicroscopic Structure of the Inner Ear*. Pergamon Press, London.
- Kimura R S, Schuknecht H F & Sando I 1965

- Fine morphology of the sensory cells in the organ of Corti of man. *Acta Otolaryngol* (Stockh) 58: 390
- Kimura R. 1975 The ultrastructure of the organ of Corti. *Int Rev Cytol* 42: 173
- Kollmann J. 1898 *Lehrbuch der Entwicklungsgeschichte des Menschen*. Verlag von Gustav Fischer, Jena
- Kolmer W. 1927 Gehörorgan in Mollendorff's *Handbuch der mikroskopischen Anatomie des Menschen*. Vol. III. Springer Verlag, Berlin
- Minot C. S. 1894 *Lehrbuch der Entwicklungsgeschichte des Menschen*. Verlag von Veit & Co., Leipzig
- Murakami T. 1974 A revised tannin-osmium method for non-coated scanning electron microscopic specimens. *Arch Histol Jap* 36: 189
- Nomura Y. 1976 Innervation of the human organ of Corti. *Acta Otolaryngol* (Stockh) 82: 317
- Pujol E. & Marty R. 1970 Postnatal maturation in the cochlear of the cat. *J Comp Neurol* 139: 115
- Retzius G. 1881 *Das Gehörorgan der Wirbeltiere*. S. S. Son & Wallin, Stockholm
- Soudyn E. R. 1976 Scanning electron microscopic study of the organ of Corti in normal and sound-damaged guinea pigs. *Ann Otol Rhinol Laryngol Suppl* 29

I. Kawabata M.D.  
Dept. of Otolaryngology  
University of Tokyo  
Tokyo  
Japan

# REVERSIBLE AND IRREVERSIBLE CHANGES OF THE STRIA VASCULARIS

## *An Evaluation of the Effects of Ethacrynic Acid Separately and in Combination with Atoxyl*

Matti Anniko

*From the Department of Otolaryngology Karolinska Sjukhuset and King Gustaf V  
Research Institute Karolinska Institutet Stockholm Sweden*

(Received July 6 1977)

**Abstract** The morphological changes in the cochlea following administration of ethacrynic acid occur initially in the stria vasculans of the basal coils as an increased vascularity of the marginal cells followed by inter- and intracellular oedema in the intermediate cell layer. The combined administration of ethacrynic acid and atoxyl (individual doses) can cause irreversible damage to the cochlear hair cells and the stria vasculans. The administration of each of them separately in the same low dose did not cause hair cell degeneration or permanent morphological changes of the stria vasculans. The increased penetration of atoxyl into the cochlea is likely to occur due to the ethacrynic acid induced change in the permeability of the endolymphatic partition so that the earlier known penetration of atoxyl into the cochlea is increased.

The stria vasculans of the inner ear is of fundamental importance for the preservation of the specific composition of the endolymph, indicating a close interaction between the hair cells of the cochlea and the stria vasculans. In recent years the increase in the use of potent diuretics such as ethacrynic acid, furosemide and others in clinical work has, because of ototoxic adverse effects, focused attention to the close relationship that exists between the kidney tubules and the stria vasculans of the inner ear. Morphological studies from ethacrynic acid treated specimens have revealed a more pronounced and earlier damage in the stria vasculans than of the hair cells (Matz et al., 1969; Quick & Duvall, 1970) as also described following furosemide administration, another

potent diuretic (Quick & Hoppe 1975). Brummett et al. (1977) correlated the physiological alterations of the cochlear potentials with the ultrastructural changes in the stria vasculans during the early stages of ethacrynic acid influence on the inner ear. These changes appear to be greatly reversible as compared with the effects of atoxyl, an arsenical compound, which can cause irreversible damage of stria cells (Anniko & Wersäll 1975; Anniko 1976a).

A combination of two potentially ototoxic drugs, e.g. diuretics and aminoglycoside antibiotics, when needed may involve considerable difficulties in their clinical use. The present study elucidates morphologically the mechanisms at work in the interaction between a diuretic, ethacrynic acid, and another type of ototoxic substance (non diuretic) in this case atoxyl in their combined effect on labyrinthine structures.

## MATERIALS AND METHODS

Sixty one healthy young guinea pigs (200-300 g) without clinical evidence of otitis media and exhibiting a normal Preyer's reflex were used for the experiment. Thirty of these animals were injected with ethacrynic acid (Edecrina®,

This work was supported by grants from Karolinska Institutet and the Swedish Medical Research Council (no 12X-00720).



Table I Table illustrating Preyer's reflex change in a number of guinea pigs of the experimental group initially receiving only ethacrynic acid (EA) according to the dose

Animal no	EA (mg/kg)	Preyer's reflex (hours)										Investigated (hours after injection)
		0	1	2	4	6	12	24	48	72	96	
3	25	+	+	+	+	+						6
13	35	+	+	+	(+)	(+)						6
15	35	+	+	(+)	(+)	(+)						6
9	70	+	+	(+)	—	—						6
55	25	+	+	+	+	+	+	+	+	+		7 days
56	25	+	+	+	+	+	+	+	+	+		7 days
51	50	+	—	—	—	(+)	+	+	(+)	(+)	(+)	4 days
52	50	+	—	—	—	+	+	+	—	—	(+)	4 days
31	100	+	—	—	—	—	—	(+)	+			2 days
41	100	+	—	—	—	—	—	—	+	+	+	4 days
39	200	+	—	—	—	—	—	(+)	+			2 days

\* Injection of 25 mg/kg of ethacrynic acid

\* Injection of 70 mg/kg of atoxyl

MSD) and 31 were treated with a combination of ethacrynic acid and atoxyl (Pro Gen® Sodium, Abbott Laboratories Ltd). The control group consisted of 5 untreated guinea pigs.

The animals treated with ethacrynic acid only were given a 1% solution of ethacrynic acid in isotonic saline solution subcutaneously (s.c.) as a single dose, 10–200 mg/kg b.w. The presence or the absence of Preyer's reflex was recorded every hour during the first 12 hours and thereafter every sixth hour until sacrificing the animal (Table I).

Guinea pigs treated with a combination of ethacrynic acid and atoxyl, a 2% solution in sterile water were injected s.c. with atoxyl

(50–70 mg/kg b.w.) either before simultaneously with, or following the administration of ethacrynic acid (Tables III–IV).

Following decapitation of the animal the labyrinth was perfused with 2% osmic acid as a fixative for 2 hours, rinsed in sodium phosphate buffer (pH 7.2–7.4) whereafter the microdissection was performed to separate the cochlea from the rest of the labyrinth. The specimens were dehydrated in increasing concentrations of alcohol and embedded in Epon. Light microscopic sections were stained with toluidine blue, whereas the ultrathin sections for electron microscopy were stained with uranyl acetate and lead citrate.

Table II Table illustrating Preyer's reflex change in a number of guinea pigs following administration of ethacrynic acid and atoxyl at the same point in time

Animal no	EA (mg/kg)	A (mg/kg)	Preyer's reflex (hours)										Investigated (hours after injection)
			0	1	2	4	6	12	24	48	72	96	
25	35	70	+	+	—	—							4
26	35	70	+	+	+	+							4
35	100	100	+	—	+	+							4
38	100	100	+				—	—	(+)				24
40	100	100	+				—	—	—	—			48
53	50	70	+		—	—	—	—	—	—	—	—	24
54	50	70	+		—	—	—	—	—	—	—	—	96
57	25	70		+	+	+	+	+	+	—			48
58	25	70		+	+	+	+	(+)	—	—	—		72
61	25	50		+	+	+	+	(+)	(+)	(+)			48

\* Renewed injection of 25 mg/kg of ethacrynic acid

Table III Table illustrating Preyer's reflex change in a number of guinea pigs following administration of ethacrynic acid 1-2 days before the injection of atoxyl

Animal	EA (mg/kg)	A (mg/kg)	Preyer's reflex (hours)								
			0	1	2	6	12	24	48	72	96
8	25	70	+	+	+	+	+	+	+	+	+
9	25	70	+	+	+	+	+	+	+	+	+
1	50	70	+	-	-	(+)	+	+	(+)	(+)	(+)
2	50	70	+	-	-	(+)	+	+	-	-	(+)
3	50	70	+	-	-	-	+	+	(+)	(+)	+

Injection of atoxyl

Time of sacrifice

## RESULTS

## Ethacrynic Acid

Morphological changes of the inner ear were limited to the stria vascularis and the outer hair cells without involving the neighbouring spiral ligament, spiral prominence, or Reissner's membrane. The structural changes were extensive in the animals which received the highest doses, exceeding 60-70 mg/kg, and they started in the basal part of the cochlea. An amount of 35 mg/kg (single dose) did not cause any structural alteration in the stria vascularis during the first 2 hours after its administration although Preyer's reflex became impaired during this time. In specimens studied 4-6 hours after the administration of the ethacrynic acid, a mild intercellular oedema could sometimes be observed in the intermediate cell layer of the stria vascularis of the basal coil. A normal ultrastructure was present in specimens treated with a dose less

than 35 mg/kg (subcutaneously) and also in those which were investigated 2-7 days following the administration of 10-60 mg/kg.

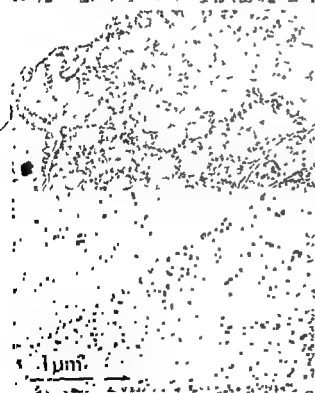
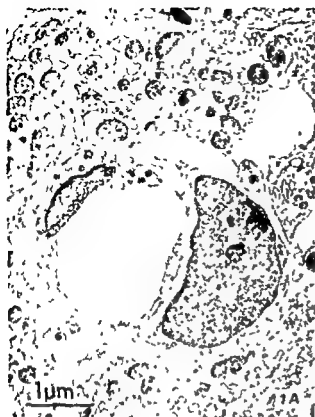
With an increasing amount of ethacrynic acid ( $\geq 70$  mg/kg) stria changes were also observed when the specimens were investigated only  $\frac{1}{2}$ -1 hour following the subcutaneous injection. The changes after this time were usually restricted to the 1st and the 2nd coils but 2-4 hours following the ethacrynic acid administration all coils were morphologically altered. Specimens investigated 24-48 hours after the injection with the same amount of ethacrynic acid showed less extensive changes in the stria vascularis than those observed after only 2-5 hours thus indicating a reversible process.

The structural changes of the stria vascularis followed the same basic pattern in all investigated specimens. Initially an increased thickness was observed in the light microscope. The electron microscope showed a

Table IV Table illustrating Preyer's reflex change in a number of guinea pigs following administration of ethacrynic acid 3-24 hours after the injection of atoxyl

Animal	EA (mg/kg)	A (mg/kg)	Preyer's reflex (hours)								
			0	1	2	6	12	24	48	72	96
11	35	70	+	+	+	+	+	+			
12	35	70	+	+	+	(+)	(+)	+			
13	50	100	+	+	+	+	+	+			
14	50	100	+	+	+	+	+	+	(+)	+	+
15	50	100	+	+	+	+	+	+	(+)		

\* Administration of ethacrynic acid  
 † Time of sacrifice



mild intercellular oedema in the intermediate cell layer, beginning around the stria blood vessels (Fig. 1). The marginal cells contained an increased number of cytoplasmic vesicles (Fig. 2) but also the mitochondria were affected early showing a slight swelling and disorganization of their internal structure (Fig. 3). Similar mitochondrial changes were also found in the intermediate cells. Later, an increase of the intercellular spaces and a vacuolization of both the marginal and the intermediate cells occurred. However, the marginal cells were less affected than the intermediate cells, although both cell types revealed a shrinkage of the cell volume during the late stages of the changes (Fig. 4). The melanin granules normally contained within the intermediate cells were resistant to atrophy. However, melanin particles were often found in the pericellular space and many of them were in contact with the surface of the marginal cells and formed imprints in the cytoplasm (Fig. 3b) as if they had been ejected from the marginal cells. No morphological changes were discernible in the capillary endothelium of the blood vessels and the basal cells always remained normal. In spite of the sometimes pronounced changes of the stria vascularis (Fig. 4) the lumen of the marginal cells facing the endoneurial space always remained smooth without disrupting the junctions between the cells. The morphological changes of the stria vascularis appeared greatly reversible. The alterations registered 24–48 hours follow-

ing the treatment were less pronounced than those observed only a few hours following the ethacrynic acid injection. The organ of Corti appeared intact even when corresponding parts of the stria vascularis were morphologically changed.

### Combinations of Ethacrynic Acid and Atoxyl

#### Administration of ethacrynic acid and atoxyl simultaneously

The morphological changes were closely correlated to the change in Preyer's reflex (impairment or total loss). No considerable structural alteration of the stria vascularis was observed in animals with a preserved Preyer's reflex.

Simultaneous administration of ethacrynic acid ( $\geq 50$  mg/kg) and atoxyl (70 mg/kg) caused a rapid and mostly a persistent loss of Preyer's reflex. The morphological changes of the stria vascularis were, however, frequently less pronounced than those following only ethacrynic acid treatment after the same length of time, except for the rejection of some stria cells from the surface of the stria vascularis mainly close to the spiral prominence (Fig. 5a–b). In these specimens only a mild intercellular oedema occurred. Most rejected cells were recognized as marginal cells showing vesiculation of the cytoplasm but containing many quite well preserved mitochondria. The hair cells of the organ of Corti were in these specimens ultrastructurally normal at the adjacent level of the stria vascularis. Afferent nerve endings could appear swollen but efferent nerve endings were morphologically unchanged.

The combined administration of ethacrynic acid and atoxyl often resulted in a prominent change in the mitochondrial features, showing osmophilic inclusion bodies adjacent to the mitochondrial cristae. These inclusions were found both in mitochondria with a preservation of cristae and in those in various stages of degeneration with fragmentation of the internal structure (Fig. 6).

Fig. 7. Electron micrograph of a marginal cell.

Fig. 8. Electron micrograph of a marginal cell.

Fig. 9. Electron micrograph of a marginal cell. Increased number of cytoplasmic vesicles in a marginal cell. Stria vascularis. Early stage of ethacrynic acid-induced changes. (A) In the marginal and the intermediate cell extensions mitochondria often reveal a slight swelling of the cristae. An oedema is present between the cells. (B) Melanin particles in close contact with the apical cell surface as if they were expelled into the scala media.

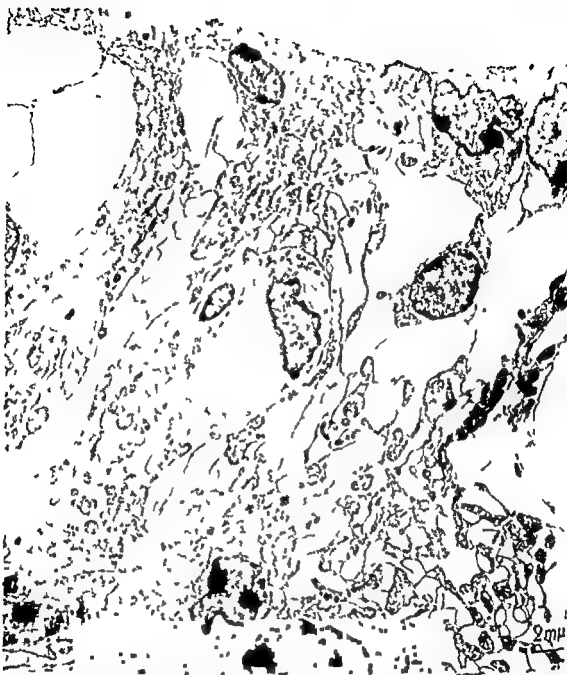


Fig 4 EM Severe oedema of the marginal and intermediate cell layers of the stria vascularis following the administration of ethacrynic acid. The basal cells appear

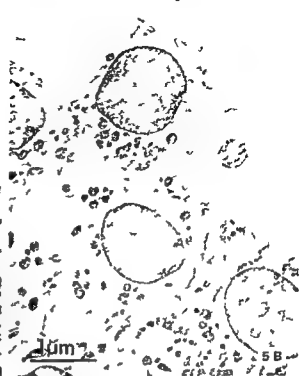
morphologically intact. The marginal cell cytoplasm contains a great number of small vesicles.

Fig 5 EM Stria vascularis. Combination of ethacrynic acid and atoxyl. Damaged stria cells are expelled into the endolymphatic space.

Fig 6 EM Stria vascularis. Combination of ethacrynic acid and atoxyl. Marginal cell osmiophilic inclusion

bodies (arrows) are frequently found in morphologically preserved cells.

Fig 7 EM Combination of ethacrynic acid and a stria vascularis. Osmiophilic debris in the region between intermediate and basal cells.



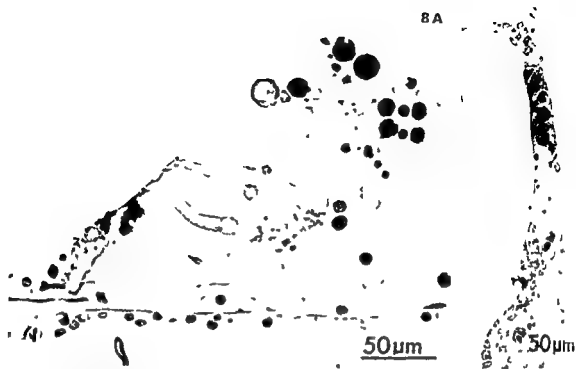


Fig 8 LM Combination of ethacrynic acid and atoxyl (A) Organ of Corti. Oedema in the cytoplasm of the outer hair cell in the first row (B) Stria vasculans at the

adjacent level. Protrusion of stria cells into the endolymph

In specimens investigated shortly (1–12 hours) following the treatment, the organ of Corti appeared mainly unaffected. If the animals were sacrificed 1–4 days later the outer hair cells were either severely degenerating (vesicular degeneration) or their place had at least in part been filled with proliferating supporting tissue. The inner hair cells too were morphologically altered.

A divergence from the normal position of Reissner's membrane could appear following the combination of ethacrynic acid and atoxyl. During the first 12 hours after the administration of the drugs it often showed a hydrops labyrinthine. After 1–2 days the membrane sometimes appeared depressed over the degenerating organ of Corti. Specimens investigated 4 days following the treatment could show a normal position or a depression of Reissner's membrane. However the hair cells had in these cases degenerated completely and were replaced by scar tissue.

The effect of ethacrynic acid alone is mainly

exerted on the basal coils of the cochlea in contrast to the atoxyl effect appearing primarily in the apical part of the inner ear. Three guinea pigs were injected with 25 mg/kg of ethacrynic acid and 50 mg/kg of atoxyl. The specimens were investigated after 24 hours and showed a normal ultrastructure of the stria vasculans in the two basal coils but in the third coil marginal cells protruded into the endolymphatic lumen (Fig 8a–b). The administration of separate but identical doses of ethacrynic acid and atoxyl does not affect the stria vasculans. Multivesiculated osmiophilic bodies were sometimes found among the stria cells (Fig 7) in the late stage of ethacrynic acid induced change. The organ of Corti appeared mostly normal in these cases, though a mild oedema in some outer hair cell could occur.

*Administration of ethacrynic acid before atoxyl*  
Pretreatment with ethacrynic acid renders the inner ear more vulnerable to the toxic

effect(s) of atoxyl. A single dose of 50 mg/kg ethacrynic acid and 70 mg/kg of atoxyl did not damage the hair cells of the cochlea when administered separately, whereas their combined effects, although administered with an interval up to 12–18 hours, can cause irreversible damage to the hair cells and an increase in the number of cells in the corresponding parts of the stria vascularis.

#### *Administration of ethacrynic acid*

##### *Experimental*

A single injection of 35–50 mg/kg of ethacrynic acid 4–6 hours following the administration of 70 mg/kg of atoxyl did not cause morphological changes in the cochlea when the specimens were investigated 4–7 hours following the last injection, although Preyer's reflex was absent when the investigation was made 1–3 days later when the reflex had returned, hair cells and the stria vascularis were normal.

## DISCUSSION

The clinical reports by Pillay et al (1969) and Schwartz et al (1970) of ototoxic effects due to ethacrynic acid focused attention to the dangers that may arise following the use of potent diuretics which interfere with ion and water transport through plasma membranes. The structural and physiological similarities between the stria vascularis of the inner ear and the kidney tubules are well established and hereby received additional confirmation.

The results of the present investigation reveal the time course relationship of ethacrynic acid. It has a rapid effect on the cochlea and may cause impairment or loss of hearing. However these effects are—at least initially—reversible. Ethacrynic acid induced ototoxicity has been reported by several investigators (Matz et al, 1969; Quick & Duvall, 1970; Ernstsosson, 1972; Silverstein & Beggs, 1974; Matz, 1976) but whether or not this drug can produce permanent hearing loss in patients with normal renal function is still not

settled. An association between impaired renal function and the ototoxicity of this drug has been shown under controlled laboratory conditions in the guinea pig (McCruddy et al, 1974). Ethacrynic acid induced damage is also reported with the concurrent administration of an aminoglycoside antibiotic (West et al, 1973; Kaku et al, 1973).

Structural changes of the secretory epithelium may also be induced experimentally by means of atoxyl administration (Anniko & Wersäll, 1975, 1977). However low dosages of this drug caused primary morphological damage to the hair cells at the apical part of the guinea pig inner ear (Anniko, 1976b). Whereas the ethacrynic acid induced changes of the stria vascularis is reversible in the early stage of toxic reaction, the comparative changes found after atoxyl intoxication are mainly of irreversible type.

The combination of ethacrynic acid and atoxyl has synergistic effects in their affection of cochlear hair cells which may be severely damaged while the corresponding parts of the stria vascularis appear morphologically less affected or even completely normal. The administration of each substance separately in the same low dose did not, however, cause hair cell degeneration or persistent morphological changes of the stria vascularis. The rejection of stria cells is probably an elimination process of irreversibly damaged biological material. The intramitochondrial inclusion bodies may consist of accumulations of atoxyl but a degenerative transformation of the mitochondrial structure cannot be excluded. Since many toxic substances act through a common morphological pathway of degeneration (Trump & Ginn, 1969) the appearance of intramitochondrial inclusions early during the cellular degeneration may indicate a primary site of toxic damage.

Recent results by Bosher (1977) indicate that ethacrynic acid acts in three main phases. Initially a rapid and short lasting (20–40 minutes) inhibition of active transport processes occurs followed by a delayed and long lasting (about



12 hours) mitochondrial dysfunction. Also a reduction in the overall permeability of the endolymphatic system takes place, lasting about 12 hours. However, tertiary effects arising from these alterations may furthermore cause an overall permeability change. The ethacrynic acid induced change in the permeability of the endolymphatic partition is likely to increase the earlier known penetration of atoxyl into the cochlea (Anniko & Plantin, 1977) because of a probable dysfunction of the serum endolymph barrier of the inner ear (decreased threshold level for inflow to the cochlea?). Such a mechanism would explain the clinical findings of a rapid affection of hearing following ethacrynic acid administration during treatment with aminoglycoside antibiotics (Matz et al., 1969).

Both ethacrynic acid and atoxyl can affect the metabolism of endolymph as demonstrated by morphological changes in the stria vascularis. Comparison of the electrophysiological response to ethacrynic acid and atoxyl (Cohn et al., 1974; Leonard et al., 1971; Borscher et al., 1973; Prazma et al., 1974) shows that both cause depression of the cochlear microphonics (CM) and the direct current potential (EP). Whereas the CM and the EP showed almost complete recovery within one hour of ethacrynic acid administration, the effects of arsacetin (acetylated atoxyl) persisted for a longer time. Arnold et al. (1976) reported that the EP decreased significantly when atoxyl induced morphological changes appeared in the stria vascularis and that the physiological and morphological alterations appeared almost simultaneously concerning the time course relationship.

The effects of atoxyl at the cellular and subcellular level were analyzed by Thelestam et al. (1977) reporting a direct damage to the plasma membrane (holes) but also an impairment of the protein metabolism occurred intracellularly.

Kuypers & Wilberts (1976) described how ethacrynic acid affects the ATP production intracellularly and concluded that this may

explain the occurrence of a wide variety of changes that appear at various metabolic levels in the living cell due to ethacrynic acid.

A combination of ethacrynic acid and atoxyl may impair or even totally block the endolymph metabolism for so long time that the ionic balance is altered and becomes toxic for the hair cells. In addition to the suggested increase in atoxyl penetration into the inner ear, an impairment of the atoxyl elimination from the endolymph via the stria vascularis may also occur, thus exposing the cochlear hair cells to toxic concentrations of atoxyl over prolonged period of time.

In clinical work it is of importance to consider the possibility of toxic interaction when combining two or several potentially ototoxic drugs, even though they may have different site(s) and mode(s) of action at the cellular/subcellular level and in doses which when administered separately, do not cause ototoxic effects.

## ZUSAMMENFASSUNG

Nach Verabreichung einer Dose von Ethakrynsäure zeigen sich die morphologischen Veränderungen in der Cochlea zuerst in der Stria vascularis der Basalmembran als eine zunehmende intrazelluläre Blasenbildung in der Stria vascularis. Die gleichzeitige Anwendung von Ethakrynsäure und Atoxyl (einmalige Dosen) kann unwiederumliche Schädigungen an den cochleären Haarzellen und der Stria vascularis verursachen, während ihre separate Anwendung in gleichen schwachen Dosen keine Degeneration der Haarzellen und keine bestehenden morphologischen Veränderungen bewirkt.

## REFERENCES

- Anniko M. 1976a Surface structures of stria vascularis in the guinea pig cochlea. Normal morphology and atoxyl induced pathologic changes. *Acta Otolaryngol* (Stockh) 82: 343-353.
- 1976b The cytochrome c oxidase in atoxyl treated guinea pigs. *Acta Otolaryngol* (Stockh) 82: 70-81.
- Anniko M. & Plantin L.-O. 1977 Delayed elimination of the ototoxic compound atoxyl from the inner ear. *Arch. Oto-Rhino-Laryngol* 215: 81-89.

- Anko M & Wersall J 1975 Damage to the stria vascularis in the guinea pig by acute atoxyl intoxication. *Acta Otolaryngol* (Stockh) **80**: 167-179.
- 1977 Experimentally (atoxyl)-induced ampullar degeneration and damage to the macula utriculi. *Acta Otolaryngol* (Stockh) **83**: 429-440.
- And N Chou J T Thorn L & Schinko I 1976 Surface alteration of the stria vascularis after treatment with ethacrynic acid and atoxyl: control measurements of EEC potential. Congress Abstract XIIIth Workshop of Inner Ear Biology, Dusseldorf, W. Germany.
- And S M 1977 Ethacrynic acid ototoxicity as a general model in cochlear pathology. *Adv Oto-Rhino-Laryngol* **22**: 81-89.
- And S A Smith C & Warren R L 1973 The effects of ethacrynic acid upon the cochlear endolymph and stria vascularis. *Acta Otolaryngol* (Stockh) **75**: 184-191.
- Arnsperg C R Smith C A Ueno Y Cameron S & Koshier R 1977 The delayed effects of ethacrynic acid on the stria vascularis of the guinea pig. *Acta Otolaryngol* (Stockh) **83**: 98-117.
- Ata E S Gordes E H & Brusilow S W 1971 Ethacrynic acid effect on the composition of cochlear fluids. *Science* **171**: 910.
- Brusilow S 1972 Cochlear physiology and hair cell population in a strain of the waltzing guinea pig. *Acta Otolaryngol* (Stockh) Suppl. 297: 1-19.
- Chen Y Farmer J C Jr & Hudson W R 1973 Otolotoxic drug effects on cochlear histochemistry. *Arch Otolaryngol* **98**: 282-286.
- Knapp W & Wilberts D P C 1976 The effect of ethacrynic acid on ATPase activities in the inner ear of rat and guinea pig. *ORL* **38**: 321-327.
- Leard J E Nakashima T & Snow J B 1971 The effects of damage to the organ of Corti and the stria vascularis on endolymphatic cationic changes. *Arch Otolaryngol* **94**: 541-547.
- Mez G J 1976 The ototoxic effects of ethacrynic acid in man and animals. *Laryngoscope* **86**: 1065-1086.
- Matz H J Beal D D & Krames L 1969 Ototoxicity of ethacrynic acid. Demonstrated in a human temporal bone. *Arch Otolaryngol* **90**: 157-155.
- McCrudy J A Jr McGormack J G & Harnill J A 1974 Ototoxicity of ethacrynic acid in the anurid guinea pig. *Arch Otolaryngol* **100**: 143-147.
- Pillay V K D Schwartz F D Amin K & Kark R M 1969 Transient and permanent deafness following treatment with ethacrynic acid in renal failure. *Lancet* **i**: 77-79.
- Prazna J Browder J F & Fischer N D 1974 Ethacrynic acid ototoxicity potentiation by kanamycin. *Ann Otol* **83**: 111-118.
- Quick C A & Hoppe W 1975 Permanent deafness associated with furosemide administration. *Ann Otol* **84**: 94-101.
- Quick C A & Duvall A J 1970 Early changes in the cochlear duct from ethacrynic acid: an electron microscopic evaluation. *Laryngoscope* **80**: 954-965.
- Schwartz F D Pillay V K G & Kark R M 1970 Ethacrynic acid: its usefulness and untoward effects. *Am Heart J* **79**: 427-428.
- Silverstein H Beggs R 1974 Ethacrynic acid—its reversible ototoxicity. *Laryngoscope* **84**: 976-989.
- Thelestam M Anniko M & Mollbrä R 1977 Cytopathogenic effect of atoxyl, an ototoxic compound on human diploid fibroblasts in vitro. *Chemico-Biological Interactions* **19**: 47-60.
- Trumph H F & Ginn F L 1963 The pathogenesis of subcellular reaction to lethal injury. *Meth Ach ex Exp Pathol* **4**: 1-29.
- West B A Brummett R E & Hines D L 1973 Interaction of kanamycin and ethacrynic acid. *Arch Otolaryngol* **98**: 32-37.

Matti Anniko M D  
Department of Otolaryngology  
Karolinska sjukhuset  
S 10401 Stockholm  
Sweden

12 hours) mitochondrial dysfunction. Also a reduction in the overall permeability of the endolymphatic system takes place, lasting about 12 hours. However, tertiary effects arising from these alterations may furthermore cause an overall permeability change. The ethacrynic acid induced change in the permeability of the endolymphatic partition is likely to increase the earlier known penetration of atoxyl into the cochlea (Anniko & Plantin, 1977) because of a probable dysfunction of the serum endolymph barrier of the inner ear (decreased threshold level for inflow to the cochlea?). Such a mechanism would explain the clinical findings of a rapid affection of hearing following ethacrynic acid administration during treatment with aminoglycoside antibiotics (Matz et al, 1969).

Both ethacrynic acid and atoxyl can affect the metabolism of endolymph, as demonstrated by morphological changes in the stria vasculans. Comparison of the electrophysiological response to ethacrynic acid and atoxyl (Cohn et al, 1974; Leonard et al, 1971; Bosher et al, 1973; Prazma et al, 1974) shows that both cause depression of the cochlear microphonics (CM) and the direct current potential (EP). Whereas the CM and the EP showed almost complete recovery within one hour of ethacrynic acid administration, the effects of arsacetin (acetylated atoxyl) persisted for a longer time. Arnold et al (1976) reported that the EP decreased significantly when atoxyl induced morphological changes appeared in the stria vasculans and that the physiological and morphological alterations appeared almost simultaneously concerning the time course relationship.

The effects of atoxyl at the cellular and subcellular level were analyzed by Thelestam et al (1977) reporting a direct damage to the plasma membrane (holes) but also an impairment of the protein metabolism occurred intracellularly.

Kuypers & Wilberts (1976) described how ethacrynic acid affects the ATP production intracellularly and concluded that this may

explain the occurrence of a wide variety of changes that appear at various metabolic levels in the living cell due to ethacrynic acid.

A combination of ethacrynic acid and atoxyl may impair or even totally block the endolymph metabolism for so long time that the ionic balance is altered and becomes toxic for the hair cells. In addition to the suggested increase in atoxyl penetration into the inner ear, an impairment of the atoxyl elimination from the endolymph via the stria vasculans may also occur, thus exposing the cochlear hair cells to toxic concentrations of atoxyl over prolonged period of time.

In clinical work it is of importance to consider the possibility of toxic interaction when combining two or several potentially ototoxic drugs, even though they may have different site(s) and mode(s) of action at the cellular/subcellular level, and in doses which when administered separately do not cause ototoxic effects.

## ZUSAMMENFASSUNG

Nach Verabreichung einer Dose von Ethakrynsäure zeigen sich die morphologischen Veränderungen in der Cochlea zuerst in der Stria vasculans der Basengänge als eine zunehmende intrazelluläre Blasenbildung in den Marginalzellen, welcher inter- und intrazelluläre Öde in der mittleren Zellschicht folgt. Später kann es zu einer Verdrängung der Zellen kommen.

Bei gleichzeitiger Verabreichung einer schwachen Dosis von Atoxyl und Ethakrynsäure zeigen sich keine Veränderungen in der Stria vasculans, sondern nur eine Verdrängung der Zellen in der mittleren Zellschicht. Es ist wahrscheinlich, dass die Kombination der beiden Substanzen zu einer stärkeren Schädigung der Stria vasculans führt.

## REFERENCES

- Anniko M. 1976a Surface structure of stria vascular in the guinea pig cochlea. Normal morphology and atoxyl induced pathologic changes. *Acta Otolaryngol* (Stockh) 82: 343-353.
- 1976b The cytocochleogram in atoxyl treated guinea pigs. *Acta Otolaryngol* (Stockh) 82: 70-81.
- Anniko M & Plantin L O. 1977 Delayed elimination of the ototoxic compound atoxyl from the inner ear. *Arch Otorhino Laryngol* 215: 81-89.

Tabelle I Zelldichte im Ganglion spirale (Zell/cm<sup>2</sup> 0.01 mm<sup>2</sup>)

	n	M	s
Vermutete			
Windung 1 (basal)	118	32.70	4.56
Windung 2	100	33.09	4.73
Windung 3	91	30.15	3.30
Windung 4 (apikal)	29	31.01	3.56
Streptomycinbehandelte Tiere			
Windung 1 (basal)	260	30.99	7.11
Windung 2	201	29.56	6.04
Windung 3	253	27.15	5.28
Windung 4 (apikal)	111	24.47	5.56

Die Zelldichte wurde in n Zellen auf 0.01 mm<sup>2</sup> berechnet. Die Mittelwerte der Zelldichten sind in der Tabelle mit M angeschrieben. Die Unterschiede zwischen normalen und mit Streptomycin behandelten Tieren erwiesen sich als statistisch signifikant. Die Abnahme der Ganglienzelldichte in unseren Fällen ist wesentlich geringer als die von Ostyn u. Thylberg (1968) beschriebene, die nach 30 Tagen Neomycingabe in der Basalwindung 25-75% betrug. Die stärkste Abnahme mit über 90% zeigte sich in der Spitzenwindung. In den übrigen Windungen lag die Abnahme zwischen 3 und 10%.

## DISKUSSION

Die Deutung der dargelegten Ergebnisse ermöglichen soll kurz auf bisher publizierte Wirkungen ototoxischer Antibiotika in der Cochlea eingegangen werden. Im Bereich der Haarzell-Lagers wird von den meisten Autoren (z.B. Kohonen 1965, Engström 1966, Spoendlin 1966, Stupp et al. 1973, Wersall et al. 1973) anfangs ein Zugrundegehen der äußeren Haarzellen, basal beginnend, im weiteren dann ein Untergang der inneren Haarzellen apikal beginnend beschrieben. Offensichtlich erfolgt anschließend die Degeneration der zum Ganglion spirale führenden Nervenfasern (Kohonen 1965, Johnson 1974). Da durch die Untersuchung von Spoendlin (1973) bekannt ist, daß die

überwiegende Zahl der Ganglienzellen den inneren Haarzellen zugeordnet ist, muß angenommen werden, daß nach Zugrundegehen der inneren Haarzellen mit einer stärkeren Degeneration von Ganglienzellen zu rechnen ist. Dies wurde auch von Ylikoski et al. (1974) nachgewiesen. Der in unserer Versuchsreihe beobachtete Abfall der Ganglienzelldichte im apikalen Bereich konnte mit der Schädigung der inneren Haarzellen im apikalen Bereich zusammenhängen. Die Ausbildung der sekundären Degeneration erfordert einige Wochen, da bei Fallberichten von Patienten mit Neomycin-Intoxikation 17 Tage (Lewy 1973) bis 21 Tage (Lenhardt 1970) nach der Intoxikation postmortal noch keine Ganglienzellschaden bei völlig destruiertem Cortisches Organ nachweisbar waren. Johnson (1974) führt allerdings auch an, daß der im allgemeinen bestehende Zusammenhang zwischen Haarzellschaden und Degeneration der Spiralganglienzellen in einer kleinen Zahl von Fällen nicht beobachtet wurde. Es besteht also die Möglichkeit, daß primär die Nervenfasern degenerieren, ohne daß das Cortische Organ geschädigt ist. In ähnlicher Weise wird von Bredberg (1973) ein Fall von symmetrischer Lärmschwerhörigkeit beschrieben, bei dem es zu einem beidseitigen Ausfall der äußeren Haarzellen, aber nur zu einer einseitigen Nervenfaserdegeneration in der Lamina spiralis ossea kam. Für die Erklärung der Degeneration von Spiralganglienzellen bei Einwirkung ototoxischer Antibiotika wäre also auch eine direkte Schädigung der Nervenzellen zu diskutieren. Die Untersuchungen von Stupp et al. (1965), Voldrich (1965) und von anderen Arbeitsgruppen haben ergeben, daß parenteral applizierte ototoxische Antibiotika in der Perilymphe einen weit höheren Spiegel als in anderen Körperflüssigkeiten erreichen. Dieser hohe Spiegel bleibt sehr lange erhalten (Ilberg et al. 1974). Der Zutritt der Perilymphe zu den Strukturen des Modiolus ist in mehreren Untersuchungen näher beschrieben worden (Arnold 1974, Wicke u. Firbas 1974) und zeigt die Möglichkeit der Einwirkung toxi-

## ZUR STREPTOMYCINSCHADIGUNG DES GANGLION SPIRALE

W. Wicke, B. Welleschik, W. Firbas and H. Sinzinger

*Aus der I Hals Nasen Ohrenklinik und aus dem I Anatomischen Institut  
der Universität Wien Österreich*

(Eingegangen am 1. Mai 1977)

**Abstrakt** Es wurde die Ganglienzell-dichte im Ganglion spirale bei normalen und bei mit Streptomycin (3 Wochen 250 mg/kg täglich) behandelten Meerschweinchen untersucht. Es ergab sich eine signifikante Abnahme der Ganglienzellen mit dem Maximum der Abnahme in den Schnecken-spitzen. Als Ursache dieser Abnahme werden sekundäre Degeneration nach Haarzellverlust oder direkte neurotoxische Schädigung der Ganglienzellen diskutiert.

Die ototoxische Wirkung des Streptomycins ist seit seiner klinischen Erprobung im Jahr 1946 bekannt (Brown u. Hinshaw, 1946, Glorig u. Fowler, 1947, Hawkins et al., 1952). Während immer wieder auch eine zentrale Lokalisation der Schädigung diskutiert wird (Winston et al., 1949), steht seit den Arbeiten von Stupp et al. (1965) der periphere Angriffspunkt im Vordergrund. Stupp et al. konnten durch pharmakokinetische Untersuchungen zeigen, daß parenterale Applikation von Aminoglykosiden in Peri- und Endolymph eine hohe, wesentlich länger als im übrigen Organismus bestehende Konzentration der ototoxischen Antibiotika auftritt, die für die spezifische Organotoxizität verantwortlich ist. Als Substrat dieser Schädigung konnten morphologische Veränderungen an zahlreichen Strukturen der Cochlea beschrieben werden. Dabei erregte das Schädigungsmuster der Haarzellen das meiste Interesse. Über Zellschädigungen im Ganglion spirale bestehen einzelne Berichte (Ruedi et al., 1953, Beck 1962), jedoch ohne genauere quantitative Angaben. In den meisten morphologischen Arbeiten über die Ototoxizität wird auf das Ganglion spirale überhaupt nicht eingegangen. Lediglich bei Ostyn u. Thyberghem (1968) und Ylikoski et al. (1974) finden sich Prozent

zahlen über die Zellabnahme im Ganglion spirale. Es sollte deshalb das Ausmaß und die Lokalisation von Nervenzelluntergängen im Ganglion spirale nach Streptomycineinnahme untersucht werden.

### MATERIAL UND METHODE

Fünf junge Meerschweinchen mit einem Durchschnittsgewicht von 400 g bekamen durch 3 Wochen 250 mg/kg Körpergewicht Streptomycinsulfat (Pfizer) pro Tag subcutan injiziert. 6 Wochen später wurden die Tiere dekapitiert, die Schläfenbeine isoliert und diese nach Eröffnung der Bulla und des Vestibulum labyrinthi in 5% Formol fixiert. Nach Ylikoski et al. (1974) ist nach 4 Wochen eine der Läsion entsprechende vollkommene Degeneration von Nervenzellen zu erwarten. Die Entkalkung erfolgte in EDTA bei 5°C. Nach Einbettung in Paraffin wurden 15 µm dicke Schnittserien angefertigt. An Fotografieren wurde in der früher beschriebenen Weise (Firbas et al., 1970) die Ganglienzell-dichte bestimmt und mit der von Kontrolltieren verglichen. Die statistische Absicherung der Signifikanz erfolgte mittels *t*-Test.

### ERGEBNIS

Die Schädigung des Cortischen Organs konnte an den Schnittserien nicht exakt beurteilt werden. Es lag jedoch keine komplette Degeneration vor. Die an den Schnittserien der verschiedenen Tiere erhobene Ganglienzell-dichte ist in der Tabelle 1 zusammengestellt.

## THE INFLUENCE OF COCHLEAR TEMPERATURE ON THE ELECTRICAL TRAVELLING WAVE PATTERN IN THE GUINEA PIG COCHLEA

H H de Brey and J J Eggermont

*From the ENT Department Academisch Ziekenhuis Leiden Leiden The Netherlands*

(Received July 23 1977)

Cochlear microphonics (CM) were recorded in the guinea pig using differential recording from the first and third cochlear turns as well as by using a 10-electrode array inserted in the scala tympani of the basal turn. The maximum profile as represented by the CM was quantified by amplitude and phase data and was measured at normal temperature (38°C) and a 10° lower cochlear temperature. It appears that the CM pattern shifts toward the base of

the basilar membrane in this field. With the Mossbuer technique, Johnstone & Boyle (1967) and Rhode (1971) obtained in vivo results at high frequencies and moderately high intensities (70-90 dB SPL). Wilson & Johnstone (1972) used a capacitive probe technique and Kohlöffel (1972) used laser illumination to measure basilar membrane movements. All these investigations led to the determination of a high frequency slope for the movement of the basilar membrane of about 100 dB/octave and a low frequency slope of 10 dB/octave.

The electrical travelling waves recorded by cochlear electrodes (Eldredge et al., 1971) are thought to reflect the movement pattern of the basilar membrane. This cochlear microphonics (CM), which is variable in potential, is generated in the hair cells, and, as Dallos & Wang (1974) have shown, mainly in the outer hair cells. In guinea pigs in which the outer hair cells were selectively destroyed by the application of kanamycin in ototoxic quantities, the CM output was 30-40 dB less sensitive. The CM generated in the outer hair cells is proportional to the displacement of the basilar membrane. A relatively large quantity of quantitative data concerning the movements of the basilar membrane has been published over the last few years. Since von Békésy (1960) measured the movement and envelope of the travelling wave in cadaver ears, which required very high sound intensities (120-140 dB SPL), a number of more sensitive techniques have

been used in this field. With the Mossbuer technique, Johnstone & Boyle (1967) and Rhode (1971) obtained in vivo results at high frequencies and moderately high intensities (70-90 dB SPL). Wilson & Johnstone (1972) used a capacitive probe technique and Kohlöffel (1972) used laser illumination to measure basilar membrane movements. All these investigations led to the determination of a high frequency slope for the movement of the basilar membrane of about 100 dB/octave and a low frequency slope of 10 dB/octave. It is assumed that the tuning of the basilar membrane depends to a large extent on the value of the compliance of the membrane which changes by about a factor 100 from base to apex (von Békésy, 1960). In addition there may be a small effect of viscosity loading due to the cochlear fluid but this seems to be restricted to the very low frequencies (Zwislocki, 1953). In model experiments von Békésy (1960) observed that an increase in the viscosity of the fluid by a factor 10 caused a basal shift of about half an octave in the maximum of the travelling wave envelope. Dahl & Kleinfeldt (1973) reported an increase in the viscosity of perilymph by a factor of 2 when the temperature was lowered from 38° to 28°C. Cooling can therefore be expected to have an effect on the tuning of the basilar membrane, either by the small effect of viscosity loading of the basilar membrane or by the unknown effect on the compliance of the basilar membrane.

scher Substanzen direkt auf den Nervus cochlearis Mootz et al. (1972) beschreiben eine Schädigung der Gefäße des Plexus cochlearis nach Kanamycin-Intoxikation. Von Theopold (1976) wurde jedenfalls eine direkte neurotoxische Wirkung der Aminoglykosid-Antibiotika beschrieben. Das Schädigungsmuster läßt in unserem Fall eher eine sekundäre Degeneration nach Verlust von Haarzellen vermuten.

## SUMMARY

The neuronal density in the spiral ganglion was investigated in normal and in streptomycin-intoxicated guinea pigs. Streptomycin was administered in a dose of 250 mg/kg for 24 days. 6 weeks after the streptomycin exposure a histological examination revealed a decrease in ganglion cell density in the spiral ganglion with maximum loss in the apical portion of the ganglion. As mechanisms of the ototoxic effect a secondary degeneration of spiral ganglion cells after hair cell loss or a primary neurotoxic degeneration are discussed.

## LITERATUR

- Arnold W. 1974 Zur Frage der Produktion und Resorption der Perilymphe (Lymphabfluß des Innenohres). *Laryng Rhinol Otol* 53: 774.
- Beck Ch. & Krahel P. 1962 Experimentelle und feingewebliche Untersuchungen über die Ototoxizität von Kanamycin. *Arch. Ohr Nas KehlkHeilk* 179: 594.
- redberg G. 1973 Experimental pathology of noise induced hearing loss. *Adv. Otorhinolaryngol* 20: 102.
- Brown H. A. & Hinshaw H. C. 1946 Toxic reaction of streptomycin on the eighth nerve apparatus. *Proc. Staff Meet. Mayo Clin* 21: 347.
- Engstrom H., Ades H. W. & Andersson A. 1966 *Structural Pattern of the Organ of Corti*. Almqvist & Wiksell, Stockholm.
- Firbas W., Wicke W. & Volavsek Ch. 1970 Über Zahl und Anordnung der Ganglienzellen im Ganglion spirale des Meerschweinchens. *Mscr. Ohrenheilk. Laryng Rhinol* 104: 241.
- Glorig A. & Fowler E. P. 1947 Tests for labyrinth function following streptomycin therapy. *Ann. Otol. (St. Louis)* 56: 379.
- Hawkins J. E. jr., Rahway N. J. & Lurie M. H. 1952 The ototoxicity of streptomycin. *Ann. Otol. (St. Louis)* 61: 789.
- von Ilberg Ch., Arnold W. & Ritter R. 1974 Ursachen und Beeinflussung der Ototoxizität des Streptomycins und verwandter Antibiotika. *Laryng. Rhinol. Otol* 53: 112.
- Johnsson L. G. 1974 Sequence of degeneration of Corti organ and its first order neurons. *Ann. Otol. Rhinol. Laryngol* 83: 294.
- Kohonen A. 1965 Effects of some ototoxic drugs upon the pattern and innervation of cochlear sensory cells in the guinea pig. *Acta Otolaryngol. (Stockh.)* Suppl. 208.
- Lehnhardt E. 1970 Zur Ototoxizität der Antibiotika. *HNO (Berlin)* 18: 97.
- Lowry L. M. 1973 Acute histopathologic inner ear changes in deafness due to neomycin: a case report. *Ann. Otol. Rhinol. Laryngol* 82: 876.
- Mootz W., Schöndorf J. & Werner J. 1971 Elektronenmikroskopische Untersuchungen am Plexus cochlearis nach Kanamycin-Intoxikation. *Acta Otolaryngol. (Stockh.)* 73: 38.
- Ostyn F. & Tybergheim J. 1968 Influence of some streptomycins antibiotics on the inner ear of the guinea pig. *Acta Otolaryngol. (Stockh.)* Suppl. 214.
- Ruedi L., Graf H. & Tschirren H. 1953 Vorläufige Mitteilung über die toxische Wirkung von Neomycin auf das Gehörorgan des Meerschweinchens. *Schweiz. Med. Wochenschr* 40: 951.
- Spoendlin H. 1966 Zur Ototoxizität des Streptomycins. *Pract. Otorhinolaryngol* 28: 305.
- Spoendlin H. 1973 The innervation of the cochlea. *Ann. N.Y. Acad. Sci.* 197: 1-11.
- Stufli 11. 1973 Inner ear concentrations and ototoxicity of different antibiotics in local and systemic application. *Arch. Otol. Rhinol. Laryngol* 12: 350.
- Theopold H. M. 1976 Morphologische Untersuchungen zur Neurotoxizität von Aminoglykosidantibiotika. Elektronenmikroskopische Befunde dosisabhängig induzierter Mitochondrienschädigung im Nucleus cochlearis des Meerschweinchens. *Laryng. Rhinol. Otol* 55: 786.
- Voldrich L. 1963 The kinetics of streptomycin, kanamycin and neomycin in the inner ear of the guinea pig. *Acta Otolaryngol. (Stockh.)* 60: 243.
- Wicke W. & Firbas W. 1974 Experimentelle Untersuchungen der perilymphatischen Verbindungswege bei der Katze. *Arch. Otol. Rhinol. Laryngol* 208: 167.
- Winston J., Lewey F. H., Parenteau A., Marden P. J. & Cramer F. B. 1949 Experimental studies of streptomycin on the central vestibular system. *Ann. N.Y. Acad. Sci.* 988: 1-11.
- Ylikoski J., Wersall J. & Björkroth B. 1974 Comparative studies on the cochlear pathology and hearing loss in guinea pigs after intoxication with ototoxic antibiotics. *Acta Otolaryngol. (Stockh.)* Suppl. 326.
- Zöllner Ch. 1974 Einfluß von Neomycinsulfat auf die efferenten Sinnesaktionspotentiale des N. cochlearis beim Meerschweinchen. *Arch. Otol. Rhinol. Laryngol* 208: 227.

Univ.-Doz. Dr. W. Firbas  
I. Anatomisches Institut, Wahringerstraße 13  
A-1090 Wien, Österreich

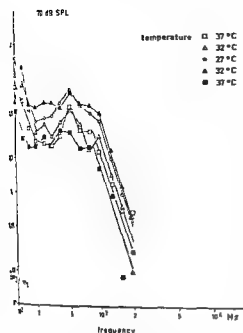


Fig. 2. The ratio of CM amplitude from the first and third cochlear turns as a function of frequency at three cochlear temperatures. The ratio is larger for lower temperatures and some hysteresis is present. The shift of the curves is in form.

laboratory type DL 102S) and an XY recorder (Hewlett Packard, type 7035B) permitting a time resolution of 0.02 msec in the recordings.

In experiment 2, the CM was recorded from the scala tympani electrodes with reference to the cervical muscles. The recorded CM was amplified 10 times, each electrode having its own pre-amplifier. All pre-amplifiers are mutual and have flat response curves from 1 Hz to 10 kHz. The pre-amplifier of the basal electrode (no. 0) is permanently connected to a main amplifier (500–25 000 times), those of electrodes 1 to 10 are successively connected to the second main amplifier. With a galvanometer the amplitudes and phase differences between electrodes 1 to 10 with respect to electrode 0 are recorded.

#### Temperature control

The animal's temperature was measured both rectally and in the oropharynx with NTC resis-

tors. The cochlear temperature was assumed to be almost the same as that of the oropharynx, but it is possible that cooling and warming of the animal leads to some lag in the cochlear temperature.

Therefore, the compound action potential latency (AP) for a high frequency tone burst was recorded before and after each series of measurements and during the cooling and warming periods. Since it is known that the AP latency is very sensitive to temperature as well as to other metabolic disturbances (Gannon et al., 1966; Eggermont, 1974) it is made a condition that the beginning and end latencies for a complete experiment be identical. Coats (1965) reported an increase in AP latency with decreasing temperature amounting to 0.06 msec/°C, while Eggermont (1974) reported a change amounting to a factor of 1.6 per 10°C temperature.

## RESULTS

### Experiment 1. Differential Electrode Recording

#### Cochlear microphonic amplitude

The CM amplitude measured in  $\mu$ V RMS was determined for 9 guinea pigs in the first and third cochlear turns at two temperatures: normal (37°C) and 10°C lower. The mean values are plotted in Fig. 1. The output of the first cochlear turn is relatively constant over the whole intensity range, whereas the third turn responds only to frequencies below 2 kHz. When the cochlear temperature is lowered the output of both turns decreases.

A convenient way to demonstrate a differential effect of cooling on the output of both cochlear turns is determination of the amplitude ratio  $CM_3/CM_1$ . This is illustrated in Fig. 2 for one animal. The temperature sequence comprises 5°C steps in the following order: 37°, 32°, 27°, 32° and back to normal. It is clear that the  $CM_3/CM_1$  ratio increases when the temperature is lowered and that this trend continues in the initial part of the rewarming period. The initial and final series are, however, almost



Under the assumption that the CM provides a representation of the tuning properties of the basilar membrane (Dallös, 1973a), we studied the influence of temperature on the electrical travelling waves in relation to possible changes in the mechanical tuning of the cochlea

## METHODS

### *Surgical procedure and electrode placement*

The data reported here are based on experiments performed in 13 guinea pigs. Premedication consisted of 15 µg atropine sulphate per kg body weight, 5 mg promethazine hydrochloride, and 5 mg chlorpromazine hydrochloride applied intramuscularly 30 minutes pre-operatively. A moderately deep anaesthesia was obtained with urethane administered intraperitoneally at a dose of 1 g/kg body weight. Tracheotomy was routinely performed, and the cochlear bulla of the right ear was exposed via the standard latero-ventral approach described by Tasaki & Fernandez (1952).

In experiment 1, nichromesteel wire electrodes with a diameter of 25 µm were introduced into the scala tympani and scala vestibuli of the first and third cochlear turns, one pair 4 mm and the other pair 14 mm from the stapes. In experiment 2, a multi-electrode array consisting of 11 electrodes about 0.2 mm apart, was inserted into a slit in the scala tympani of the basal turn according to Kohlöffel (1971). The animal was cooled according to the procedure described by Eggermont (1974) and placed in a sound-treated room which was shielded electrically

### *Stimulation, calibration, and recording*

Continuous tones and tone bursts were used as stimuli and presented via a STC-4026A dynamic headphone attached to a modified ear speculum, giving a closed sound system of about 5 cm<sup>3</sup>. With a calibrated probe microphone (Bruel & Kjaer cathode follower type 2615) and a 1/2 inch cartridge (type 4133) in combination with a probe 1.6 mm in diameter and 15 cm long the sound pressure level, at a point a

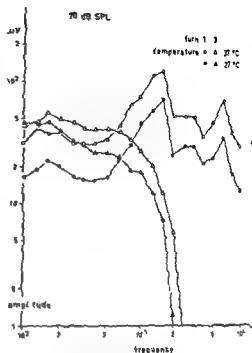


Fig. 1. The amplitude of the CM as a function of frequency for two cochlear temperatures at 70 dB SPL. Lowering of the temperature results in a decrease in amplitude.

few millimetres from the eardrum, was recorded during the experiments. The continuous tones were generated by a Rhode & Schwarz (type FTA) wave analyser with a coupled generator and passed to the headphone via a calibrated attenuator (1 dB steps). The tone bursts always started at a zero-crossing of the sine wave (for details, see Spoor, 1974).

In experiment 1, the responses recorded from the scala vestibuli and scala tympani were pre-amplified 10 times and then fed into a differential amplifier. A compensation network (Tasaki et al., 1952) was used to separate the AP and SP from the CM. The CM output was measured after a total amplification of 10 to 25 000 times, using the wave analyser with 1 Hz band width. Phase measurements were performed, without filtering, with a gain-phase meter (Hewlett-Packard, type 3575A). For measurements of travelling wave delay, the CM response to low-frequency tone bursts was recorded with the use of an averager (D.

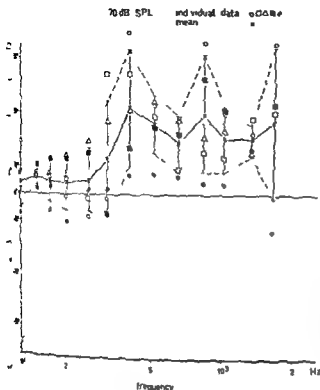


Fig. 4. The increment in phase difference between the first and third cochlear turns due to cooling as a function of frequency. In the middle frequency an average increase of 24 degrees difference is observed after lowering the temperature by 10°C.

35°C and 28°C. Consistent results were obtained in 4 animals.

A representative case is shown in Fig. 5 (a), (b) for various stimulus frequencies.

The phase data for the lowest frequency (1 kHz) can serve as control, since at this frequency the wavelength of the travelling wave will be about 10 mm, which is large with respect to the width of the electrode array (2 mm). Consequently, little phase variation is to be expected and the amplitude distribution should be uniform due to the lack of phase interaction.

With increasing frequency, the CM amplitude decreases for all electrodes in the array progressively toward the more apical electrodes. The phase gradient across the electrode array increases progressively with stimulus frequency leading to phase reversal (e.g. at 13 kHz the phase difference exceeds 180°).

Cooling causes no systematic changes in the phase difference at low frequencies. For high frequencies the phase difference increases,

which is in accordance with a basal shift of the travelling wave profile. From Fig. 6 it is quite clear that around 13 kHz for all electrodes there is a shift of the phase reversal of the order of 250 Hz due to the cooling. As Fig. 5 (a, b, c) shows, the CM amplitude decreases as a result of cooling and in conformity with the results of experiment 1 in the basal turn. For high frequencies, i.e. 12 kHz and higher the CM amplitude increases slightly for the more apical electrodes. At 13 kHz cooling causes the phase reversal to shift from electrodes 9 and 10 to electrodes 7 and 8. The corresponding amplitude minimum is situated at the same electrodes. These results point to a basal shift of the CM pattern with one or two electrode distances (i.e. 150–300 µm).

## DISCUSSION

In experiment 1, the CM profile was studied with the use of amplitude as well as phase data. Phase differences reflect travelling wave

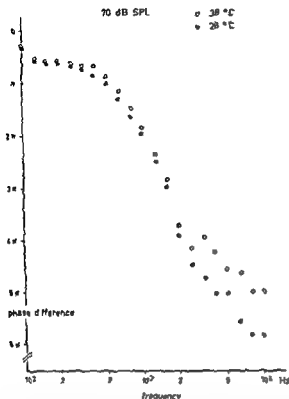


Fig. 3. Average phase difference between the CM recorded from the first and third cochlear turns. A frequency-dependent phase difference increasing from an almost constant value of  $\pi/2$  radians at low frequencies toward  $4\pi$  radians at 2 kHz is evident. As a result of cooling by  $10^\circ\text{C}$ , the phase difference increases by about  $\pi/2$  radians at a given frequency. This is thought to be caused by a shorter travelling wave length at lower temperature for the frequencies in this part of the cochlea.

the same. For the whole series of 9 animals, the average shift in frequency producing the same  $\text{CM}_2/\text{CM}_1$  output at both temperatures was calculated. This was done for  $\text{CM}_2/\text{CM}_1$  values of 1.2, 1.0, 0.5, and 0.2. It was found that the frequency shift was not systematically dependent on the segment of the curves under consideration, indicating a uniform shift of the curves as a whole. The value of this shift, which is  $1.3 \pm 0.2$ , points to an upward shift in frequency amounting to about  $1/3$  octave.

#### Cochlear microphonic phase

Between the CM differentially recorded from the first and third cochlear turns there is a phase difference. For the group of guinea pigs

under study, the average phase difference between these two turns is plotted as a function of stimulus frequency at two different temperatures in Fig. 3. At low stimulus frequencies the average phase difference amounts to  $\pi/2$  radians, increasing up to  $4\pi$  radians at 2 kHz. Values above 2 kHz are probably due to remote contributions, and must be considered with reserve.

Lowering of the temperature by about  $10^\circ\text{C}$  results in an increase of the phase difference at a given frequency amounting to about  $\pi/2$  radians. This is visualized in Fig. 4, which shows the individual phase differences in ears of the 9 animals together with the mean value and standard deviation (area between dashed lines). It is evident that in general there is an increase in phase difference which amounts to about  $5^\circ$  up to 300 Hz and then rapidly increases and stays at a value of about  $20^\circ$  up to 2 kHz. Values above 2 kHz have been omitted. For the region above 500 Hz, there was an increase in phase in all but one case.

#### Electrical travelling wave delay

When stimulation is performed with short bursts, the CM recorded from the first and third cochlear turns shows a characteristic lag in onset, amounting to between 0.65 and 1 msec, depending on the interelectrode distance. A series of measurements was performed with tone bursts of various frequencies below 1 kHz at the normal temperature and below the normal temperature.

For 6 guinea pigs the average lag was  $0.9 \pm 0.1$  msec at  $37^\circ\text{C}$ . For the  $10^\circ$  lower temperature the lag increased slightly and amounted to  $0.9 \pm 0.1$  msec, giving an average shift of msec ( $p < 0.005$ ).

#### Experiment 2. Multi-electrode Array Recording

The amplitude and phase distribution of the CM is measured along the electrode array, at stimulus frequencies ranging from 1–14 kHz at a level of 90 dB SPL for cochlear temperature

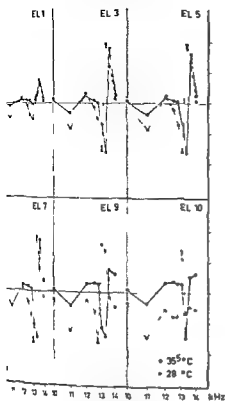


Fig. 6. The CM phase as a function of frequency at different temperatures. Cooling causes an increase in phase difference in accordance with a basal shift of around 12.

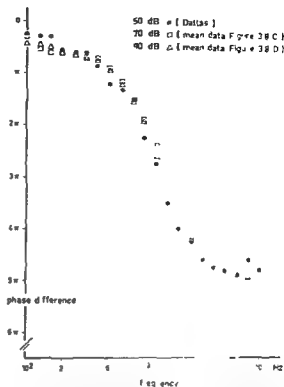


Fig. 7. The phase difference between the CM recorded from the first and the third cochlear turn. The frequency-dependent accumulating phase was obtained in experiments at normal body temperature in agreement with the phase difference calculated from experiments by Dallos (1973b).

phase difference between the first and third cochlear turns and compared the result with our experimental data (Fig. 7), which showed marked agreement. On the basis of the basal shift Dallos calculated the travel time of CM from the first to the third cochlear turn to be about 1.1 msec. In our direct measurements a value of 0.8 msec was obtained. The increased travel time for the lower temperature corresponds to a larger phase difference than was measured directly. A larger phase difference for a given stimulus frequency corresponds to a basal shift of the maximum of the travelling wave envelope. The problem is that the amplitude data and the phase data show opposite effects. Since, as already stated, the metabolism of the hair cells is influenced by the cochlear temperature, the temperature coefficient may have been different

for the first and third cochlear turns. This is illustrated in Fig. 8 where the temperature coefficient for the CM recorded in both turns at 70 and 90 dB SPL is plotted (averaged data for 9 guinea pigs). Here the temperature coefficient is about 0.5 for the first turn and about 0.9 for the third turn, at least for sufficiently low frequencies. The temperature coefficient in the third cochlear turn appears to be frequency dependent, which points either to remote contributions from the higher turns since the temperature coefficient drops to the first turn value, or else to a frequency dependent non-linearity that is also temperature dependent.

These problems did not arise in experiment 2, where all the recordings are from the basal turn and show a conformity between amplitude and phase data in that a cooling of about

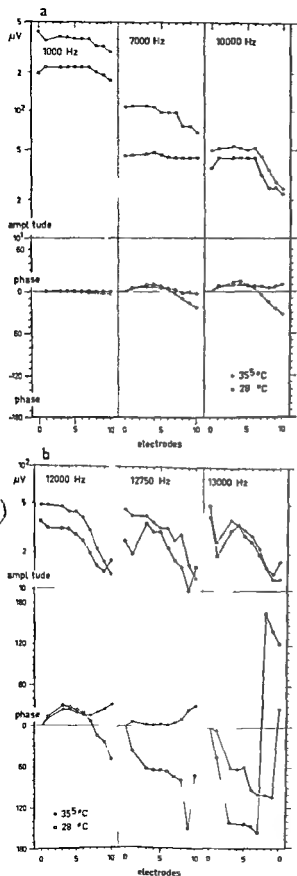


Fig 5 (a-c) The CM amplitude (in  $\mu V$  RMS) and the phase gradient along the electrode array at various frequencies and different temperatures at 90 dB SPL. Cooling leads to an increase in phase difference and a shift of the phase reversal along the electrode array. This is in accordance with a shift of the CM excitation pattern of about 250  $\mu m$ .

delays and tuning characteristics of the basilar membrane while amplitude data also bear witness to the state of the CM generators and the metabolism of the hair cells. From the amplitude data it may be concluded that the amplitude profile shifts toward the apex when the temperature is lowered. However, the phase shift and travelling wave delays are consistent with a shift toward the base of the cochlea. Thus there seems to be a contradiction.

Dallos (1973b) measured the phase shift between the differentially recorded CMs from the first, second and third cochlear turns and sound at the eardrum for an intensity of 50 dB SPL. From his data we calculated the cumulative



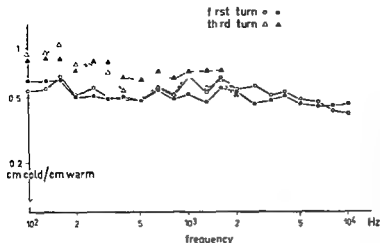


Fig 8 The average ratio of the CM recorded at low and normal function of frequency. For the first turn the ratio is frequency independent, amounts to 0.5. For the third turn the ratio is frequency dependent and is about 0.5 at low frequencies, which indicates a different effect of temperature in the first and third turns.

8°C produces a basal shift of the excitation pattern along the cochlear partition. This basal shift means that the compliance of the basilar membrane increased when the temperature was lowered. Although this may sound a bit unphysical at first, because one expects cooling to make the basilar membrane stiffer, the results agree with those obtained by Eatock & Manley (1976), whose recordings from single auditory nerve fibres in the gecko showed that the characteristic frequency of the fibre increased when the temperature was raised. This indicates an apical shift of the stimulation profile under an increase in temperature.

The increase in viscosity of the perilymph (Dahl & Kleinfeldt, 1973) will not have any influence on the phase data according to Zwillock's (1953) theory of cochlear mechanics, but may influence the amplitude distribution, especially in the apex of the cochlea. The effect will be a slight basal shift of the travelling wave envelope maximum.

The smallness of the effects on the mechanical tuning produced by cooling of the cochlea suggests that some counteracting effects might be responsible for these results. On the basis of two independent sets of measurements it remains difficult, however, to consider other mechanisms besides the increased compliance and increased viscosity responsible for the effects observed.

## ZUSAMMENFASSUNG

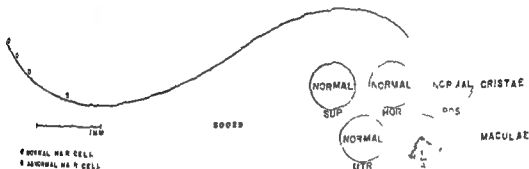
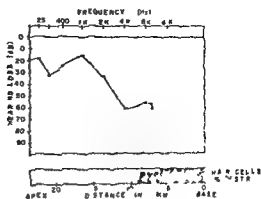
Mikrophonpotentiale wurden von der ersten und dritten Windung der Schnecke bei 16°C und 8°C registriert.

Es zeigt sich, daß die Amplitude der Erregung einer bestimmten Reizfrequenz in der ersten Windung frequenzunabhängig ist, während sie in der dritten Windung frequenzabhängig ist.

Die Ergebnisse deuten auf einen unterschiedlichen Effekt der Kühlung der Schnecke auf die ersten und dritten Windungen hin.

## REFERENCES

- Bekesy von G 1960 *Experiments in Hearing* McGraw-Hill New York
- Coats A C 1965 Temperature effects on the auditory apparatus *Science* 150 1481
- Dahl D & Kleinfeldt D 1973 Die Viskosität der Lymphe in Abhängigkeit von der Temperatur. In *Funktion und Therapie des Innenohres* (ed H Jakobi, P Lots) Johann Ambrosius Barth Leipzig p 164
- Dallos P 1973a *The Auditory Periphery* Academic Press New York
- Eggermont J J 1974 The temperature dependent cochlear adaptation and masking in the guinea pig *Audiology* 13 147
- Eldredge D H, Benitez L D & Tempier J W 1974 Electrical travelling waves in the cochlea of the chilla. In *Physiology of the Auditory System* (ed B Sachs) National Educational Consultants Baltimore
- Gannon R P, Lazlo C A & Moscovitch D H 1974 Late effects of kanamycin intoxication *Audiology* 13 27



There was damage to the ... of the saccule which wave ...

ultrasound literature provides more describing clinical findings than data regarding the effect of ultrasound upon the auditory and vestibular systems. Long term follow-up studies do appear to indicate improvement with ultrasound (Stahle, 1976). However there must be crucial differences, for as (1973) pointed out, the results of ultrasound therapy vary considerably among surgeons. Certain data is available regarding the effects of ultrasound on sensory elements, supporting structures, and endolymphatic systems (Portmann et al, 1952, Braun et al, 1960, McLay et al, 1961, Formby, 1963, ... et al, 1971, Crysdale & Stahle, 1971).

Little behavioral experimental data has been published regarding the round window approach. McGee et al (1963) have focused on the effect of direct stimulation of the cochlea by a relatively high dose of ultra-

sound. However little experimental data is available on the effects of the currently used levels of ultrasound upon the auditory function using the round window technique.

The purpose of the present study was to investigate the effect of ultrasound irradiation presented through the round window with emphasis upon auditory effects of treatment. Sound-conditioned cats were used to allow evaluation of functional changes in the auditory system. The levels of ultrasonic irradiation approximated those levels at present being used with the round window approach (Basek, 1970, Kossoff 1972).

## METHODS AND PROCEDURES

Thirteen healthy adult animals were used in the experiment. These comprised 2 sham audiometric control animals, 2 sham histological control animals and 9 experimental ani-



## ULTRASONIC IRRADIATION THROUGH THE ROUND WINDOW

### *Functional and Morphological Findings in Sound conditioned Cats*

K R Bouchard and J T Benitez

*From the Division of Otolaryngology William Beaumont Hospital Royal Oak Mich USA*

(Received June 15 1977)

**Abstract** Ultrasound was presented through the round window in 9 sound-conditioned cats at levels approximating clinical usage Threshold shifts were mild to moderate and confined to 4 kHz and higher There was total loss of function at 16 kHz in 2 animals Threshold shifts correlated with cochlear histological findings as shown by reconstruction There were three main types of lesions abnormalities of supporting structures hair cell loss and lesion of Reissner's membrane Supporting structure damage was the most frequent The cristae of the semicircular canals did not show any lesions though some saccular damage was noted These changes were not observed in 4 control animals Some conductive damage was noted as a result of probe placement High frequency loss can be expected with the round window approach at 10 radiation levels of 52 mW for 20 min or stronger

The use of ultrasound has become increasingly widespread as a treatment for Meniere's Disease Ironside & Lindsay (1959) have pointed out that ultrasound had been used for the treatment of otosclerosis as early as 1928 Reports of treatment for various ear disorders had also appeared in 1948, though with considerably varying results (Arslan, 1953) The use of ultrasound has been pursued more extensively since Krejci described in 1949 applying it to the vestibule (Anagnò, 1960)

The purpose of using ultrasound, for Meniere's symptomatic treatment, was to eliminate the vestibular part of the labyrinth while attempting to maintain hearing function The semicircular canal approach had been used almost exclusively for clinical treatment until the mid sixties Much of the development and clinical experience can be ascribed to Arslan (1953), Krejci (Anagnò 1960), James (1965, James et al 1960) among others Kossoff & Khan (1966) suggested that an improved technique would in itself be advantageous A

method of directing the ultrasonic beam directly through the round window was presented as that alternative (Kossoff et al 1967)

It was felt that the round window approach eliminated several disadvantages of the semicircular canal approach Some of these were unnecessary mastoidectomy, potential risk hearing loss, possible facial nerve damage and inefficient transmission of ultrasonic wave The round window approach provides a more direct (primarily fluid) pathway for the ultrasound irradiation This is important since the absorption coefficient is much higher for bone than it is for fluid In approaches requiring transmission of the ultrasonic waves through bone (e.g. semicircular canal approach) considerable energy is absorbed by the bone and dissipated in the form of heat In early experiments this heat itself became a problem during ultrasonic presentation

Since the introduction of the procedure using the round window, several investigators have presented clinical findings Some compare the round window vs other approaches (Arslan, 1968, Basek, 1973) Others provide evidence that tends to establish the advantages of the round window approach (Kossoff et al, 1968, Basek, 1970, Kossoff, 1972)

Study conducted in part Otolaryngological Research Laboratory Wayne State University School of Medicine Partially supported by a grant from the Deafness Research Foundation

Findings were reported in part at the XV Pan American Congress of Oto-Rhino-Laryngology and Broncho-Entomology in New Orleans Louisiana November 1976

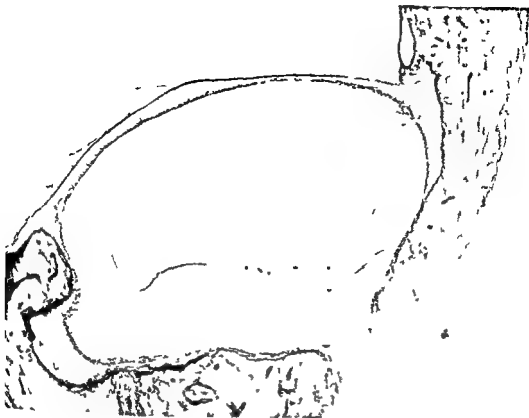


Fig. 1. Photomicrograph of the round window which shows thickening of the round window membrane with exudate partially blocking the niche in the animal described in Fig. 1. Hematoxylin-eosin  $\times 52$ .

Eleven animals underwent preliminary colectomy (Paparella & Hohmann, 1962) labyrinthectomy followed by audiometric and-conditioning and threshold determination (Culler et al., 1935, Schuknecht, 1953, Ott et al., 1960). The histological sections underwent threshold

Their

tracheotomy was performed and a small tube was attached with silk sutures to allow adequate ventilation. A bulla approach was performed and adequate exposure obtained with a selfretaining retractor. The mucosa was elevated from the bulla and a small shell of bone was removed by drilling around close to the margins of the bulla.

The bulla was filled with distilled water. The ultrasound probe was placed in the round window niche and held by a mechanical support. Bleeding was minimal, if blood did block round window, it was gently aspirated and distilled water again applied.

Ultrasonic irradiation was presented via ultrasonic stimulator using a 11 mm transducer in a 15 mm diameter probe. A similar sized sham probe for all control animals. The apparatus previously described by Kos



A



B

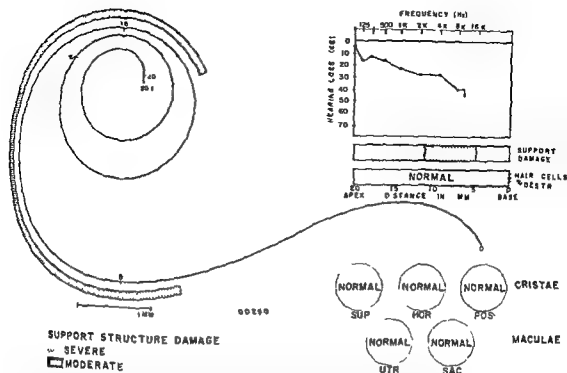


Fig 5 Cochlear reconstruction of a medium level animal showing supporting structure damage from 4.25 mm to 1 mm along the cochlea. This corresponds to frequency

hearing losses seen from 2 kHz to 16 kHz. The hair cell population is normal. No abnormalities are seen in the vestibular labyrinth.

off et al (1967). It was not necessary to irritate the bulla during irradiation. Kossoff (1972) has established that with this apparatus at the low level of ultrasound irradiation used, temperature rise was not sufficient to be destructive. The levels of stimulation and irradiation times were low (approximately 1 mW/10 min), medium (approximately 54 W/20 min), and high (approximately 110 W/20 min). Since monitoring of the output level is not possible, it is essential to perform calibration before and after application. A method of calibration described in the ir-

radiator manual was found to be satisfactory. The output is measured by observing weight changes with a sensitive analytical balance. The levels determined pre- and post-operatively were usually quite close ( $\pm 0.5$  mW). However, actual output did not correspond to the manual settings on the equipment.

At the end of the appropriate stimulation, the distilled water was gently aspirated and probe removed. The shell of bone was replaced and sutured with wire. The skin and subcutaneous tissues were approximated and sutured. Tracheostomy tube was removed. Clinical observations were made post-operatively, as were repeated threshold determinations, approximately 2 weeks, one month, and 2 months post-operatively. Intravital perfusion was performed and temporal bones removed and prepared for processing (Schuknecht, 1953).

Fig 4 (A) Photomicrograph of the macula of the saccule showing moderate hair cell loss in the sensory epithelium.





Fig 7 Photomicrograph of the posterior crista showing normal sensory epithelium in the same medium level animal reconstructed in Fig 5 Hematoxylin-eosin  $\times 240$

## FINDINGS

### Functional findings

There were minimal clinically observable vestibular alterations after ultrasound irradiation.

Fig 6 (A) Photomicrograph of a normal organ of Corti in the animal shown in Fig 5 at 14.5 mm along the cochlea. The supporting structures can be seen firmly attached to the basilar membrane. (B) Photomicrograph of the organ of Corti at the 8 mm region of the cochlea in the same animal. The supporting structures are pulled away from the basilar membrane leaving the Böttcher's cells (B). The hair cells are normal. Hematoxylin-eosin  $\times 450$ .

In 4 animals, there was slight nystagmus towards the operated ear for 2–3 days post-operatively. However, there were no symptoms that would suggest labyrinthectomy.

Audiometric functional findings can be summarized as follows:

A. Animals that showed no significant change (within 10 dB of threshold). This group included the audiometric control animals and 2 other cats, one that had received low irradiation and one that had received medium. A slight improvement was noted between the first post-operative audiometric testing and

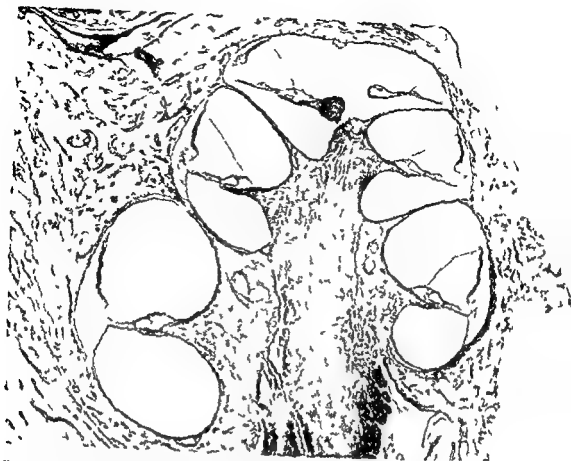


Fig 8 Photomicrograph of the cochlea from a high level irradiated animal. The Reissner's membrane is missing in the basal turn only. Hematoxylin-eosin  $\times 32$

weeks) and the final post operative testing (2 months) in the control animals

**B** Two animals showed moderate threshold shifts throughout post operative testing which was greatest for the lower frequencies. Both were of the low irradiation group. One of them had a quite marked progressively deteriorating threshold at all tested frequencies. This animal was found to have otitis media.

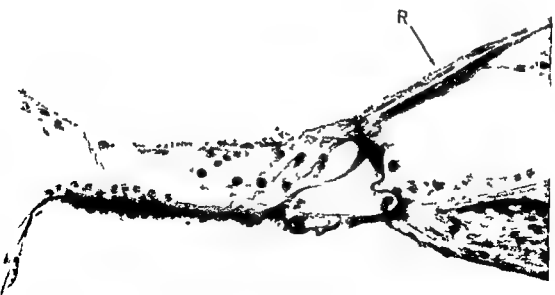
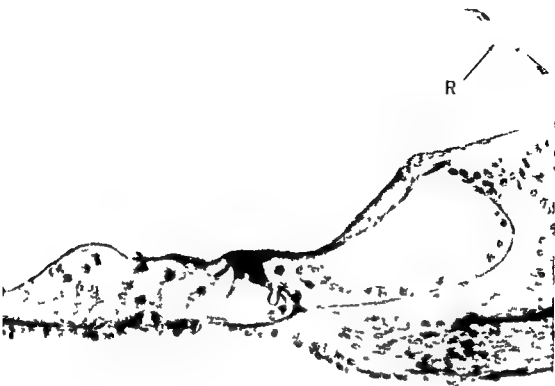
**C** Two animals showed slight to moderate high frequency shifts (one medium and one high level). In these cats the high frequency shift stayed rather stable throughout the post operative testing. In one there was an improvement in the lower frequencies similar to that noted in the control animals.

**D** Three animals showed moderate to severe shift of threshold values in high frequencies (2 medium and one high level). Two

exhibited total loss at 16 kHz. Two animals showed some shift in the threshold value at the lower frequencies as well. In one cat (medium level) the threshold further deteriorated after the first post operative session, marked at the high frequencies. However, other 2 animals showed rather stable high frequency losses.

Thus, the audiometric findings indicated that the medium to high level animals a high frequency shift more noticeable from 4 kHz to

Fig 9 Photomicrograph showing the comparison of normal position and collapsed Reissner's membrane in cochlea of the animal shown in Fig 8. (A) Normal position of Reissner's membrane at the 15 mm region (R). The lapse of the tectorial membrane is an artifact seen throughout the cochlea. (B) Reissner's membrane (R) is above the tectorial membrane extending across the hair cells in the 8 mm region. Hematoxylin-eosin  $\times$





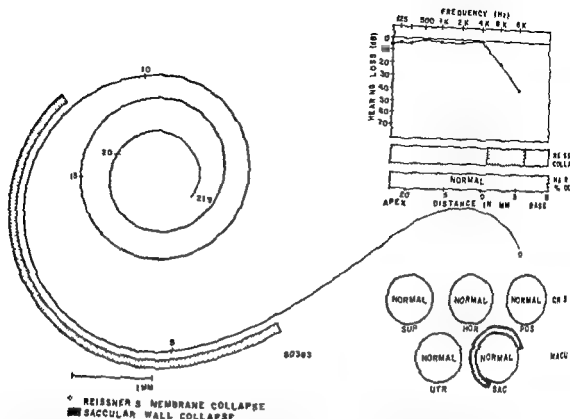


Fig 10 Cochlear reconstruction in the high level animal shown in Figs 8 and 9. The rupture and collapse of Reissner's membrane extends from 4 mm along the cochlea to 9.3 mm. This corresponds to the hearing loss at 8 kHz.

and 16 kHz. The saccular wall also shows collapse tending over 60% of the macular surface. The hair cells were normal, as was the rest of the vestibular labyrinth.

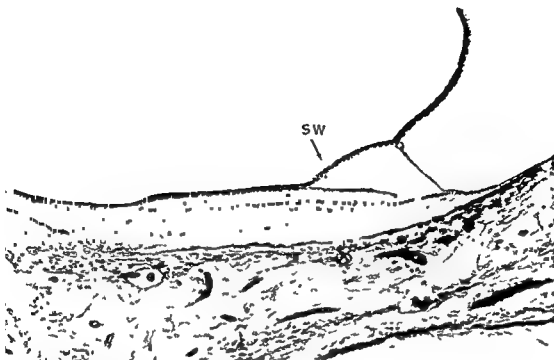
**kHz.** All losses for the low irradiation animals appeared to be conductive in nature. There were some conductive shifts in the medium and high animals as well. These conductive changes had a tendency to improve, as seen in the control animals. There was a high frequency loss due to the ultrasonic irradiation. However, the medium level and the high level dosages used in the present experiment showed no difference based on functional findings.

#### Histopathological findings

A survey of the histological findings of the ultrasound irradiated animals showed three basic types of damage. These were actual hair cell loss, abnormalities of supporting structures of the organ of Corti, and lesion of Reissner's membrane. Representative animals from

each group will be discussed along with associated vestibular histology.

**A Hair cell loss.** Fig 1 shows a cochlear reconstruction and audiogram for a medium animal (52.2 mW/20 min) that showed actual hair cell loss. There is a lesion of the organ of Corti consisting of loss of outer hair cells beginning at the lower basal turn and extending towards the hook of cochlea where the loss includes inner hair cells. The hair cell loss responds to the high frequency threshold shown in the audiogram. Neuron population in the spiral ganglion was normal throughout. There is a loss in the lower frequencies well, which may be a result of blockage of round window. There was also moderate (about 20%) of hair cells in the macula of saccule. Fig 2 makes a comparison between supporting structures at about 8 mm region.



II Photomicrograph of the saccular wall (sw) over macula of the saccule in the cat shown in Fig. 10. toluidine eosin  $\times 120$

cochlea with a more apical turn at the mm region. In 2B, the loss of outer hair cells can be seen, whereas in 2A, all hair cells are present. The outer hair cells were destroyed more apically than were the inner hair cells which only showed damage, extremely basally and toward the hook. Fig. 3 shows the round window in this animal. The membrane is severely thickened, with the round window niche showing fine granular exudate partially blocking the window. Fig. 4 illustrates the damage to the macula of the saccule. There is moderate hair cell loss in the sensory epithelium (4A), with rupture of the saccular wall inferiorly (4B).

**B Abnormalities of supporting structures** Fig. 5 is the cochlear reconstruction of another medium level animal (53 mW/20 min). There was no hair cell loss throughout. However, there was detachment of supporting structures from the spiral ligament and from the basilar membrane. The area of damage corresponds

closely to shifts noted in the final post operative thresholds. The most prominent loss occurred at 8 kHz and 16 kHz. It is interesting to note that the shift at 2 kHz and 4 kHz was not apparent until one month after irradiation. The mild losses from 62.5 to 2 kHz correspond to moderate severe thickening of the round window with serofibrous fluid in the niche. Findings were normal throughout the vestibular portion of the inner ear. Fig. 6 makes comparison between the supporting structures at the 8 mm region of the cochlea (Fig. 6B) with a more apical turn at the 14.5 mm region (Fig. 6A). In the basal portion, Bottcher's cells can be clearly seen attached to the basilar membrane, while the supporting structures have become detached. Fig. 7 shows normal sensory epithelium of the crista of the posterior semicircular canal.

**C Lesion of Reissner's Membrane** Fig. 8 shows absence of Reissner's membrane in the basal turn. This was noted in a

dosage animal (110 mW/20 min). As can be seen in Fig. 9, the membrane appears to have fallen across the tectonal membrane at the 8 mm region of the cochlea (Fig. 9B) in contrast to the appearance in the 15 mm region (Fig. 9A) which is normal. The collapse of the tectonal membrane is an artifact seen throughout the cochlea. Cochlear reconstruction and final thresholds are presented in Fig. 10. The mild to moderate shift at 8 kHz and 16 kHz corresponds to the area of rupture and collapse of Reissner's membrane. In this animal, there was mild reaction at the level of the round window. This corresponds to a near-absence of shift in thresholds 4 kHz and below. There was, in addition, a rupture of the saccular wall with collapse over 60% of the macular surface. The sensory elements remained intact. The collapse is illustrated in Fig. 11.

The findings, apparently the result of ultrasonic irradiation, have corresponded closely to high frequency threshold losses. Other audiometric changes, 2 kHz and below, have consistently been correlated with changes of a conductive nature. Most common was the thickening and blockage of the round window membrane. This was noted to a varying extent in all animals, both sham and experimental. Damage within the vestibular system was minimal, usually confined to the saccule. In the 5 animals showing threshold shifts, 3 cases were a result of supporting structure damage, one of hair cell loss, and one of collapse of Reissner's membrane.

## DISCUSSION

Portions of the present experiment are comparable to other investigations. Basek (1970) was able to show, histologically, cochlear damage in the basal turn (hair cell loss), some saccular damage, and damage to the posterior cristae. Basek used an irradiation level of 30 mW/10 min, which was chosen as the lowest irradiation level in the present investigation. The low level animals in this study did

not exhibit such damage. However, he found a considerable variability ranging from minimal damage to actual hair cell loss. In the present investigation, similar damage was noted when the irradiation level was increased to 20 min at either 52 mW or 110 mW, though the area of damage was in the bottom turn of the cochlea, only one animal showing significant hair cell lesion. Most of the cochlear damage was due to abnormality of the supporting structure. The changes observed in the vestibular system were confined to the saccule. The three cristae of the semicircular canals remained remarkably intact.

Although the point of ultrasonic application was different, the behavioral functional testing was similar to that of McGee et al. (1963). By applying ultrasound irradiation directly to the basal turn of the cochlea, they caused a mainly high frequency loss that improved gradually during the course of testing, remaining poorest at the higher frequencies. The improvement seen in the present investigation was in the lower frequencies. It is felt that this might have been due to observed changes around the round window area and would be present in the surgical approach used in the McGee study. Once a high frequency loss did appear at the first post-operative test at 2 weeks, it remained quite stable. This frequency loss did show considerable variation, ranging from no change to complete loss of function at 16 kHz. Kossoff (1972) used the round window approach and similar radiation levels showed no shift in cochlear microphonics performed immediately after radiation.

Although hair cell lesion was found in only one animal, it is a very common finding with ultrasonic irradiation. It is much more notable with more intense stimulation or stimulation closer to the cochlea (Portmann et al., 1952; Braun et al., 1960; Formby, 1963; Bas, 1970). In addition, further agreement is noted with McGee et al. (1963) and Crysdale and Stahle (1972) in that the outer hair cells were more severely damaged.

The most frequently observed damage, histologically, that was correlated with significant functional shifts, was abnormality of the supporting structure. Portmann (1952) had described damage of support structures during ultrasonic stimulation, a method which applied ultrasound through the external auditory meatus. Paparella & Melnick (1967) have described a similar uplifting from the basilar membrane of the support structures into the endolymph. This was caused by direct stimulation of the ossicles with a high speed drill. It is felt that in most previous investigations, higher levels of ultrasound were used, thus causing more severe damage than the supporting structure damage found in the present study. A similar type of separation has been observed by Lundquist et al (1971) in the cristae, where sensory cells and supporting structures showed separation from the underlying stroma in the cristae.

The other finding, rupture and collapse of Reissner's membrane, was similar to the finding of McLay et al (1961) in the guinea pig. However, audiometric changes were not determined in that study. A threshold shift solely as a result of collapse of Reissner's membrane has not previously been documented. The associated collapse of the saccular wall was also quite common, especially where there was a total collapse of the membranous labyrinth due to high level ultrasound irradiation or irradiation directly to the semicircular canal (McLay et al, 1961, Formby, 1963, McGee et al 1963, Basek, 1970). Arslan (1968) has pointed out that as a result of the angle of the niche of the round window, more damage could be expected in the basal turn of the cochlea and in the saccule.

In the present investigation, there was no evidence of a deficit in the sensory epithelium of the cristae. Crysdale & Stahle (1972) have noted the rarity of complete vestibular destruction with the semicircular canal approach. However, successful use of ultrasound using the round window approach has been reported for treatment of Meniere's dis-

ease (Kossoff et al, 1967, Basek, 1970, Basek 1973). The procedure apparently does not involve total destruction, especially with the round window approach and the current used levels. Some moderate hearing loss may be expected at higher frequencies, with possible total loss at 16 kHz. Losses also may be caused by the placement of the probe in the round window niche, especially where drilling may be performed for better probe irradiation angle.

## ACKNOWLEDGEMENTS

We are indebted to Mr Yong Ku Choe for histologic preparation and to Mr Arthur Bowden for photomicrography.

## ZUSAMMENFASSUNG

Ultraschallbehandlung ähnlich derer häufig in klinischer Anwendung wurde durch das runde Fenster an neuschallkonditionierten Katzen vorgenommen. Horschwellenveränderungen waren leicht bis mäßig und beschränkten sich auf Frequenzen 4 KHz und höher. Zwei Tieren hatten totalen Funktionsverlust auf 16 KHz. Horschwellenveränderungen bezogen sich auf cochleäre histologische Befunde. Die Korrelation wurde durch Rekonstruktion bewiesen. Drei typische Hauptplatzierungen wurden beobachtet. Abnormalitäten der Stützstruktur Verlust von Sinneszellen und Lädierung von Reissner-Membran. Schädigung der Stützstruktur wurde häufigsten bemerkt. Die Cristae der halbkreisförmigen Kanäle blieben schadenfrei jedoch einiger Schaden. Sacculae wurde beobachtet. Die Veränderungen wurden in vier Kontrolltieren nicht wahrgenommen. Sondierstellung hatte Schalleitungsstörungen in einigen Tieren zur Folge. Hochfrequenzverlust ist zu erwarten bei Bestrahlung von 52 mW für 20 Minuten oder länger durch das runde Fenster vorgenommen wird.

## REFERENCES

- Anagno R 1960 Treatment of Meniere's disease with ultrasound. *Laryngol* 429
- 1968 Ultrasonic selective irradiation of the ear windows as a new treatment of vertigo and tinnitus. *Acta Otolaryngol* (Stockh) 65: 224
- Basek M 1970 Treatment of Meniere's disease with ultrasound (round window technique). *Laryngoscope* 80: 768
- 1973 Ultrasound for Meniere's disease vs. round window approach. *Arch Otolaryngol*

- Brain D Colman H Ramsen R & Ogilvie R 1960 The effects of ultrasound on the internal ear: A histological investigation *J Laryngol Otol* 74 628
- Cody D 1973 Meniere's disease: conservative surgical therapy *Adv Otol Rhinol Laryngol* 19 314
- Crysdale W & Stahle J 1972 Ultrasonic irradiation of the guinea pig cochlea *Ann Otol Rhinol Laryngol* 81 87
- Culler E Finch G Girden E & Brogren W 1935 Measurements of activity by the conditioned response technique *J Gen Psychol* 12 223
- Elliott B Stein J & Harrison M 1960 Determination of absolute intensity thresholds and frequency-difference thresholds in the cat *J Acoust Soc Am* 32 380
- Formby M 1963 Ultrasonic destruction of the labyrinth *Acta Otolaryngol* (Stockh) 56 139
- Irwin W & Lindsay J 1959 Ultrasonic therapy for relief of vertigo due to Meniere's disease *Laryngoscope* 69 899
- James J A 1965 Ultrasonic therapy for hydrops *Laryngoscope* 75 1552
- James J A Dalton G Bullen M Freundlich H & Hopkins J 1960 The ultrasonic treatment of Meniere's disease *J Laryngol Otol* 74 730
- Kossoff G 1972 Safety factors of the ultrasonic round window irradiation technique *Arch Otolaryngol* 96 113
- Kossoff G & Khan A 1966 Treatment of vertigo using the ultrasonic generator *Arch Otolaryngol* 84 181
- Kossoff G Wadsworth J & Dudley P 1967 The round window ultrasonic technique for treatment of Meniere's disease *Arch Otolaryngol* 86 83
- 1968 Further experience with the round window ultrasonic techniques *Arch Otolaryngol* 88 154
- Lundquist P G Igarashi M Wersall J Guilford F & Wright W 1971 The acute effect of ultrasound on the ampullar sensory epithelium of the guinea pig *Acta Otolaryngol* (Stockh) 72 181
- McGee T Cole G & Van Den Ende H 1963 Effect of quantified ultrasonic sound on labyrinthine structures in animals *Laryngoscope* 73 683
- McLay K Finn M & Ormerod F C 1961 Histological changes in the inner ear resulting from the application of ultrasonic energy *J Laryngol Otol* 75 345
- Paparella M & Hohmann A 1962 Surgical technique for otological and auditory research *Ann Otol Rhinol Laryngol* 71 203
- Paparella M & Melnick W 1967 Stimulation deafness. Ch 32 in *Sensorineural Hearing Processes and Orders* (ed A Graham) Little Brown & Co Boston
- Portmann G Portmann M & Barbe J 1952 Etude expérimentale (fonctionnelle et histologique) de l'effet des ultrasons sur l'audition *Acta Otolaryngol* (Stockh) Suppl 100 119 (Belton translation 1956)
- Schuknecht H 1953 Techniques for study of cochlear function and pathology in experimental animals *Otolaryngol* 58 377
- Stahle J 1963 Some effects of ultrasound on the ear: Morphological and functional studies on domestic pigeon *Acta Otolaryngol* (Stockh) Suppl 192 191
- Stauffer R 1968 ——— of Meniere's disease
- A R Bouchard M A  
Div of Otoneurology  
William Beaumont Hospital  
3601 W 13 Mile Road  
Royal Oak MI 48072 USA

## OPTOKINETIC AFTERNYSTAGMUS AND POSTROTATORY NYSTAGMUS IN SQUIRREL MONKEYS

M. Igarashi, M. Takahashi and J. L. Homick<sup>1</sup>

*From the Department of Otorhinolaryngology and Communicative Sciences  
Baylor College of Medicine, Houston, Texas, USA*

(Received February 27, 1977)

**Abstract** Regardless of whether the direction of optokinetic stimulus matches or conflicts with that of vestibular stimulus, if the squirrel monkey subject has a good target pursuing ability, the enhancement or inhibition of poststimulatory nystagmus occurs. The nature of the stimuli to provoke optokinetic afternystagmus and postrotatory nystagmus are not the same, but the similarity of response characteristics and cross interaction strongly suggest the existence of a common neural linkage between the stimulus receptor and reactor.

The prime function of the oculomotor system is to precisely pursue the moving target in order to obtain proper visual information. In this regard, the test for eye tracking activity or optokinetic nystagmus (OKN) test is considered to be natural and appropriate manner to evaluate the oculomotor function.

When the optokinetic stimulus ceases and the subject is kept in the dark, the eyeball continuously beats to the same direction of that of OKN. This is the optokinetic afternystagmus (OKAN). Even though some characteristics are similar for OKAN and postrotatory nystagmus (PRN) (Kornhuber 1962, Takemori 1974, Robinson 1975), many things should be studied regarding the nature and characteristics of this OKAN.

Both the PRN and the OKAN may be considered undesirable phenomena to the body because of the fact that those represent prolonged poststimulatory reactions. Thus, it is suspected that the equilibrium system intends to inhibit those.

It has been well known that visual and vestibular systems coordinate each other under natural circumstances and for the purpose of smooth operation of the oculomotor (and body equilibrium) system. From this viewpoint, this present study was initiated in order to compare the physiological meanings of these two nystagmus and interaction between the PRN and the OKAN.

### METHODS

The experimental subjects were six randomly selected adult male or female squirrel monkeys with body weight range 500-700 g. The monkey was secured in the laboratory built restrainer. Undue pressure to the posterior neck region was avoided. Subdermal platinum needle electrodes were implanted at the outer canthi and the horizontal eye movement was recorded on the Beckman Dynograph through a DC amplifier. All tests were begun about 15 minutes after the administration of amphetamine 0.5 mg/kg.

The squirrel monkey was placed at the center of a 60 cm diameter illuminated white cylinder which had 16 vertical black stripes (1.7 cm wide each and equally separated). This cylinder could be rotated 0-200°/sec speed with

<sup>1</sup> Neuroscience Laboratory, Medical Research Branch, Medical Sciences Division, NASA Johnson Space Center, Houston, Texas, USA.

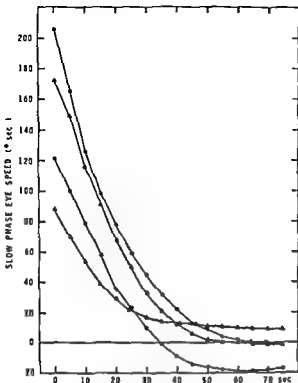


Fig 1 This figure exhibits the average nystagmic slow phase eye speed after 200°/sec stimulus in vertical axis (V 6) along the poststimulus time course (in horizontal axis) by comparing four experimental situations open circle PRN+OKAN reverse direction open triangle PRN closed circle OKAN+PRN and closed triangle OKAN

0°/sec<sup>2</sup> constant angular acceleration. This optokinetic stimulator provided a whole visual field rotation around the subject. When the stimulus speed reached 200°/sec, the illumination was instantaneously eliminated.

The rotation table at the coaxial center of the optokinetic cylinder could also be rotated at the identical speed with the same angular acceleration. When the rotatory stimulus reached 200°/sec the table was stopped (the experimental situation 2) and the illumination was abolished simultaneously (the experimental situation 3). However, this impulsive cessation of rotatory stimulus required about 1 sec to reach 0°/sec, the complete stop. The mechanical reason for this was basically due to the mass which had to be stopped.

The experimental situation 1 was the illuminated optokinetic cylinder rotation, optokine-

tic stimulus application and the recording OKN and OKAN.

The experimental situation 2 was the subject rotation in darkness, rotatory stimulus application and the recording of PRN.

The experimental situation 3 was the subject rotation in light. In other words, rotatory stimulus and optokinetic stimulus were given concomitantly in order to evaluate the coexistent condition of OKAN+PRN.

The experimental situation 4 was also subject rotation in light; however, in this instance a mirror was installed in a 45° plane in front of the squirrel monkey's eyes in order to reverse the direction of optokinetic stimulus. Therefore, in this experimental situation, the direction of rotatory stimulus and that of optokinetic stimulus conflicted.

In this experimental series, two steps of maximal speeds were studied: 100°/sec and 200°/sec.

Test trials were spaced more than three days; therefore, the identical test situation appeared once every two weeks. We repeated this test sequence three times in order to evaluate the repeatability of the results.

## RESULTS

### A Results obtained after 200°/sec stimulus

Fig 1 exhibits the average nystagmic slow phase eye speed (%/sec, vertical axis) declining as a function of time after 200°/sec stimulus along the poststimulus time course (sec, horizontal axis) by comparing experimental situation 1 OKAN (closed triangle), 2 PRN (open triangle), 3 OKAN+PRN (closed circle), and 4 PRN+OKAN reverse direction (open circle).

Experimental situation 1 (OKAN). The average slow phase eye velocity immediately after the stimulus cessation was 93°/sec. The inter-individual variance was relatively small initially, but this became larger as the nystagmus decayed. The 50% response reduction

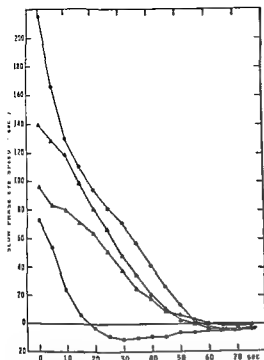


Fig 2 This figure exhibits a representation of strong slow phase eye speed reduction in OKAN+PRN situation, by comparing open circle PRN+OKAN reverse direction and closed circle OKAN+PRN. Open triangle PRN, closed triangle OKAN

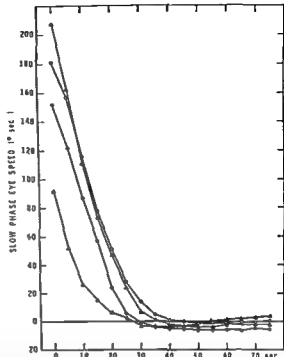


Fig 3 This figure displays a representative case which did not exhibit strong inhibition in OKAN+PRN situation. The difference between open circle (PRN+OKAN reverse direction) and closed circle (OKAN+PRN) is far less when compared to the situation displayed in Fig 2

occurred about 13 sec after the stimulus cessation

**Experimental situation 2 (PRN)** The maximum slow phase eye velocity of this PRN immediately after the stimulus cessation was 172°/sec, however it declined rapidly. Compared to the OKAN, even though the slow phase eye velocity was high, the inter-individual variance was minimal.

**Experimental situation 3 (OKAN+PRN)** The initial nystagmic slow phase eye velocity in this condition (PRN+OKAN) averaged 120°/sec. The inter-individual variance was quite large. The response declined to 50% about 15 sec after the stimulus cessation, and the curve asymptotized to a horizontal line after about 50 sec.

**Experimental situation 4 (PRN+OKAN, reverse direction)** The average slow phase eye velocity of this nystagmus response im-

mediately after the stimulus cessation was 206°/sec. The 50% response decline occurred around 15 sec after stimulus cessation.

Two representative samples of OKAN+PRN cross inhibition (experimental situation 3) are displayed (as closed circles) in Fig 2 (strong inhibition) and in Fig 3 (weak inhibition).

Under the present series of stimulus conditions, the inter-individual variance was minimal in the PRN. In the OKAN, the inter-individual variance was also minimal immediately after the stimulus cessation but it became large along the post-stimulus time course. The inter-individual variance was large in PRN+OKAN situation, but the PRN was partly inhibited by OKAN. The difference in slow phase eye speeds between PRN alone and PRN+OKAN was less than the slow eye velocity of OKAN itself (172



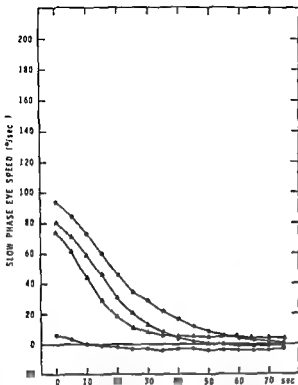


Fig 4 This figure displays the average slow phase eye speed ( $N=6$ ) after  $100^\circ/\text{sec}$  stimuli in vertical axis along the post stimulatory time span in horizontal axis under four different experimental conditions. Open circle PRN+OKAN reverse direction, open triangle PRN, closed triangle OKAN, and closed circle OKAN+PRN.

sec) The average value of slow phase eye velocity of OKAN immediately after the stimulus was  $54\%$  ( $93/172^\circ/\text{sec}$ ) of that of the PRN.

The average value of slow phase eye velocity in experimental situation 4 (PRN+reverse direction OKAN) was slightly enhanced over that of PRN itself. The difference between these two situations was smaller than the difference between the PRN and PRN+OKAN ( $206-172 < 172-120^\circ/\text{sec}$ ). Nonetheless, the result indicated that the PRN was reinforced by the existence of OKAN even when the direction was reversed.

## II Results obtained after $100^\circ/\text{sec}$ stimulus

Fig 4 demonstrates the average slow phase eye speed ( $^\circ/\text{sec}$ , vertical axis) plotted as a function of time after cessations of  $100^\circ/\text{sec}$  stimuli in four experimental situations.

**Experimental situation 1 (OKAN)** The

average slow phase eye velocity of the OKAN immediately after the stimulus cessation was  $75^\circ/\text{sec}$ . The inter individual variance was minimal, but subsequently became large along the post stimulus time course. The maximum slow phase eye speed declined  $50\%$  about 1 seconds after the stimulus cessation.

**Experimental situation 2 (PRN)** The average slow phase eye velocity of this PRN immediately after the stimulus cessation was about  $80^\circ/\text{sec}$  and the inter individual variance was minimal. The slow phase eye velocity declined  $50\%$  about 17 sec, and the curve came parallel to the zero horizontal line about 50 sec after stimulus cessation.

**Experimental situation 3 (OKAN+PRN)** The average initial value of nystagmic slow phase eye velocity resulting from this PRN+OKAN was very low, about  $6^\circ/\text{sec}$ . The inter individual variance was minimal.

**Experimental situation 4 (PRN+OKAN reverse direction)** The average nystagmic slow phase eye velocity in this reverse direction OKAN combined with PRN was  $94^\circ/\text{sec}$ . Along the post stimulus time course, inter individual variance became large. The slow phase eye velocity declined  $50\%$  around 20 sec after the stimulus termination.

Results of the OKAN (experimental situation 1) and the PRN (experimental situation 2) showed some resemblance, however, the value of slow phase eye velocity of the former was smaller and the inter individual variance was larger. In experimental situation 3 (OKAN+PRN), the post stimulatory nystagmic response was almost totally inhibited. When the reverse direction OKAN was combined with the PRN (experimental situation 4), the average value of slow phase eye velocity was slightly elevated compared to that of the PRN. The PRN and the reverse direction OKAN reinforced each other, however, the case to case variance was large, as is well displayed in Fig 5 and 6.

Fig 7 shows the nystagmic recordings after  $100^\circ/\text{sec}$  stimulus under four different stimulus situations from a representative subject.

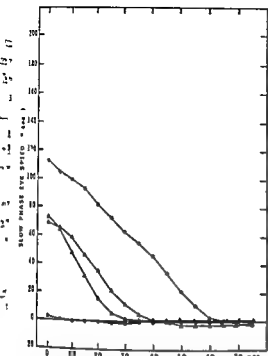


Fig 5 Similar to the situation in Fig 2 this particular subject showed strong nystagmus inhibition in the stimulus condition of OKAN+PRN (closed circle) Open circle PRN+OKAN reverse direction open triangle PRN and closed triangle OKAN

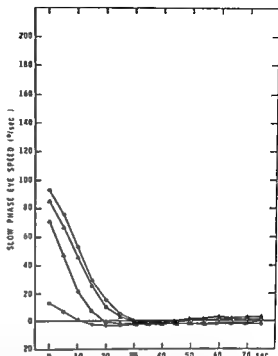


Fig 6 This figure displays less difference between the condition of PRN+OKAN reverse direction (open circle) and that of OKAN+PRN (closed circle) comparing to the results seen in Fig 5

Comparison of slow phase eye velocity of per stimulatory nystagmus elicited by subject rotation in light (experimental situation 3) and by subject rotation in light with a mirror (experimental situation 4) is exhibited in Fig 8. The figure shows clear suppression of slow phase eye velocity under the stressful situation of vestibular visual directional conflict in the latter stimulus situation. However, when a mirror was installed in a 45° angular plane in front of the subject's eyes the distance between the eyes and black stripes on white cylinder was elongated and also the visual axis and cylinder surface formed an obtuse angle causing distorted image. Even though we have used an identical stimulus speed, the true stimulus speed might have been reduced to about 60% of the original speed. The squirrel monkey subject which had the best ability to pursue reverse-direction visual target showed

54% increase to the value obtained in the PRN alone

The above described direction conflicting stimulus situation should cause very unpleasant sensation, and thus also for this reason many of the subjects might not be able to pursue visual target properly. Clearly this is the situation of vestibulo-visual conflict. An important finding in this part of the experiment was that if the squirrel monkey subject showed good reverse direction OKN even though the two stimuli are direction conflicting and unnatural, the reverse direction OKAN was positively summated with the PRN, and as a result the PRN was enhanced. This was the opposite of the results that OKAN and the PRN inhibited each other after the subject rotation in light.

The visual tracking ability was very different among the different individuals.

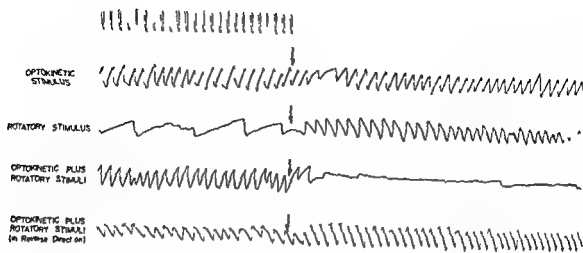


Fig. 7 Nystagmus recordings from one representative subject under four different stimulus conditions. Arrows

indicate the stimulus cessation. The maximum 100°/sec

when the system evoked the OKN (regardless of the stimulus direction) the OKAN and PRN showed similar characteristics.

### C Comparison of two stimulus conditions maximum speed—200°/sec and 100°/sec

The maximum slow phase eye velocity of the PRN immediately after the 100°/sec stimulus, was 46.5% of that which occurred after 200°/sec stimulus. Therefore, the PRN response is dependent on the maximum stimulus speed given to the peripheral cupula crista system. In contrast, the maximum slow phase eye velocity of OKAN after 100°/sec stimulus was not about 1/2 of that which occurred after the 200°/sec stimulus. The reason for this difference was that, when the maximum optokinetic stimulus speed was 100°/sec, all squirrel monkeys could pursue visual stimulus without any difficulty. However, when the maximum stimulus speed was continuously increased to 200°/sec their maximum ability of pursuit was less than 200°/sec speed. Furthermore, large inter-individual variance was observed, there were subjects with good pursuing ability and subjects with poor pursuing ability regarding the maximal stimulus speed they could pursue. The slow phase eye velocity of the OKN immediately before the termination of 200°/sec

stimulus was about 70% of 200°/sec in monkey with the best pursuing ability, its maximum slow phase eye velocity of OKAN immediately after the stimulus termination in this particular monkey was about 69% of the maximum slow phase eye velocity of PRN. Those values obtained from the squirrel monkey with the poorest pursuing ability in this group were about 41% and 45%, respectively. The maximum slow phase eye velocity of OKAN was dependent on eye speed of OKN before the stimulus cessation, and also was comparable to the eye velocity of PRN.

The overall average values indicated that the slow phase eye velocity of OKN immediately before the 200°/sec stimulus was about 53% of the stimulus speed, its slow phase eye velocity of OKAN immediately after termination of the 200°/sec stimulus was about 52% of that of PRN. If the slow phase eye velocity of OKN immediately before the stimulus termination and stimulus speed matched, the maximum slow phase velocity of OKAN immediately after the stimulus termination was almost equivalent to the maximum slow phase eye velocity of the PRN.

The squirrel monkey subjects could pursue a visual stimulus 100°/sec without

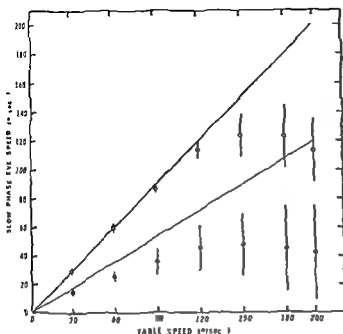


Fig 8 This figure displays the comparison of per stimulatory nystagmic slow phase eye speed elicited by rotatory plus optokinetic (open circle) and by rotatory plus optokinetic reverse direction (closed circle) stimuli. The result shows clear suppression of the slow phase eye speed under the stressful situation of vestibular visual directional conflict. The upper straight line indicates 45° line and the lower straight line is a theoretical representation of 60% of the 45° line. Vertical bars indicate standard deviations.

trouble. The average maximum slow phase eye velocity of the OKAN after 100°/sec stimulus was 75°/sec and the average maximum slow phase eye speed of the PRN after an identical stimulus speed was 80°/sec.

The stimulus condition to provoke OKAN together with PRN (the subject rotation in light) is probably the most natural stimulation condition to the body. The inter individual variance of the nystagmic response after 200°/sec stimulus in this condition was relatively large, however, it was not so after the stimulus speed of 100°/sec. After 100°/sec stimulus, these two types of nystagmus strongly inhibit each other and in most instances the slow phase eye velocity after the stimulus was less than 10°/sec.

When the stimulus is given at the maximum speed of 200°/sec OKAN was much smaller compared to the PRN. Therefore the OKAN might not influence the PRN and as a result, the difference between PRN vs OKAN + PRN was minimal. In this particular condition, the direction of OKAN and PRN were opposite. The fact that OKAN and PRN inhibited each other indi-

natures of these two types of nystagmus were similar but that these had to have some common neural linkage where the cross inhibition could take place.

Under the most natural conditions, when the body moves either actively or passively, the surrounding whole visual field moves simultaneously. In the past it has been emphasized that the inhibition of PRN is very much influenced by the visual fixation. This present experimental result (particularly after 100°/sec stimulus) indicated that if the subject pursued visual target properly, the OKAN inhibited the PRN in order to assist the nystagmic inhibition resulting from the visual fixation.

The other experimental situation, the combination of rotatory stimulus plus reverse direction optokinetic stimulus, was an unnatural situation. Comparing this PRN + OKAN (reverse direction) situation with the PRN, the difference in the slow phase eye velocity was small after 200°/sec stimulus, however, this difference was a little larger after the stimulus. Yet, these differences smaller compared to those of

In this experimental series, the table was rotated with an angular acceleration speed of  $1^\circ/\text{sec}^2$ . Under the administration of amphetamine, the majority of squirrel monkey subjects showed slight per-rotatory nystagmus in dark.

## DISCUSSION

The interaction (coordination or cross-inhibition) between the visual and vestibular systems has been known for a long time. Dodge (1923) observed that the visual fixation (or visual input) reduced the nystagmic amplitude during the body rotation with angular acceleration (per-rotatory nystagmus). By utilizing the electronystagmogram (ENG), Ohm (1926) recorded the PRN inhibition by visual fixation. These two classic observations indicated that the visual input could control the vestibular evoked oculomotor response, the former had more dominance than the latter did within the body equilibrium system.

Mowrer (1935), utilizing the pigeon, found the difference in postrotatory head nystagmus between two conditions, rotation in light and in dark. He reported that the OKAN inhibited the post-rotational vestibular nystagmus. In 1937, Mowrer further performed similar experiments in human subjects and reported that the PRN duration after the rotation in light was about 1/2 of that after the rotation in dark (His PRN observation was made in light). This observation was important because of the fact that the visual input affected the vestibular input even after the stimulus termination. Guedry et al (1961), and Collins (1968) evaluated the influence of short-term illumination on the vestibular evoked nystagmus or subjective sensation during the angular deceleration, or after the cessation of rotatory stimulus. Their observations indicated that the visual input acted in an inhibitory fashion on the vestibular input.

The functional coordination between visual and vestibular systems was reported by Fukushima et al (1975). In the rabbit, the vestibular

nystagmus was not evoked in dark with  $\text{sec}^2$  angular acceleration. However, this stimulus enhanced the provocation in this animal species.

The circularvection (the rotatory sensation provoked by rotatory optokinetic stimulation alone) was previously reported by Fick (1892), Kornmüller (1930) and Gurnee (1931). Recently Dichgans & Brandt (1972, 1973) utilized this phenomenon, successfully reproducing the pseudo-coriolis effect. Young et al (1973) studied the coordination and discoordination between circularvection and vestibular stimulation, and observed the nonlinear relationship of visual and vestibular interactions. The circularvection observed by Dichgans & Brandt (1972, 1973) and Brandt et al (1973) contributed to the understanding of the relationship between OKAN and the PRN.

The post-rotatory subjective sensation caused by cupular deviation. The degree of nystagmus and the degree of rotatory sensation are dependent on the magnitude of the vestibular stimulus, but are not necessarily equivalent to each other. When rotated in light, if the subject can pursue the target properly, the provoked OKN should have a magnitude identical to the table rotation. This optokinetic speed information interacts with the speed information delivered from the cupula cristae system. It is generally accepted that the time course of the PRN represents the degree of cupular deviation. Because of the fact that the time course of OKAN and PRN are similar, there is a possibility that the time course of PRN is also influenced by the central discharge mechanism, as Robinson (1971) pointed out.

The vestibular-visual interaction, which has been investigated through the nystagmic response or subjective sensation, became a finite phenomenon after the establishment of the direct neuronal recording at the level of the vestibular nucleus when only the visual stimulus is given (Dichgans et al, 1973; Leopold et al, 1973; Henn et al, 1974; Azzena et al, 1974). Moreover, after observing the influ-

optic stimulus at the level of the peripheral vestibular nerve in an immobilized fish. *te & Schmidt (1970)* indicated the existence of an efferent effect at the peripheral level. Those neurophysiological observations confirmed the existence of an extremely intimate visual vestibular relationship.

effects from the visual side can be detected either by visual fixation or OKN. *Fukushima & Tokita (1964)* reported that, when the direction matches the optokinetic caloric induced nystagmus enhances the other, it inhibits the other. This fact is confirmed in subhuman primates in this study. Because of the fact that those bulboculomotor response and oculomotor response were added or subtracted, these two functions should possess a common neural linkage.

*Lowrey (1935, 1937)* previously suggested a direction-dependent relationship between OKAN and PRN. Utilizing a prism he reversed the direction of optokinetic stimulus, the rotation, but failed to observe the enhancement even though the directions of OKAN and PRN were the same. In our present experiment in the squirrel monkeys, an enhancement was observed. Even though the induced angular acceleration was minimal ( $11^\circ$ ) when the visual stimulus and vestibular stimulus were given in the conflicting direction, the vestibular visual conflict occurred in 4 animals failed to pursue the visual target. However, the interindividual difference was large and when the monkeys' optokinetic target pursuing ability was good, PRN was enhanced by the OKAN.

In the present study we expected that the degree of PRN inhibition had to be dependent on the degree of OKN. Thus we have used steps of maximum speeds  $100^\circ/\text{sec}$  and  $200^\circ/\text{sec}$  to compare the effects. The interindividual variance of optokinetic target pursuing ability (maximum) in humans was reported to be large (*Blomberg 1960*). Similarly a large

inter individual variance was found in squirrel monkeys.

*Krieger & Bender (1956)* using rhesus monkeys, reported that the OKAN was inhibited when illumination was given. However, when the light was eliminated the nystagmus reappeared; therefore, this behavior of the OKAN was similar to that of the PRN. The reduction or abolition of the OKAN after brain stem lesion placement was reported by *Shanzer et al (1958)*. *Cohen et al (1971)* observed the disappearance of the OKAN after bilateral labyrinthectomy. All of these observations were done with optokinetic stimuli alone, and the interaction between the optokinetic stimuli and the vestibular stimuli was not studied.

From our present observation, the inter individual variance of the OKAN, especially regarding its duration, was relatively large. On the other hand, the intra individual repeatability of OKAN was good. The time course of OKAN and that of PRN showed a similar trend. This result suggested that the optic and vestibular after-discharge mechanisms were similar. Not only their time course but also the slow phase eye velocity of the PRN and OKAN were similar immediately after the stimulus cessation when the maximum speed was equivalent. It was thus reasonable to suspect that cross inhibition between those two stimuli could exist, specifically when the table was rotated in light and the light was eliminated when the rotation stops. This cross inhibition was clearly observed at the maximum speed of  $100^\circ/\text{sec}$ . When the maximum speed of  $200^\circ/\text{sec}$  was used, however, the subjects' target pursuing ability could not reach  $200^\circ/\text{sec}$  speed and the OKAN was not strong enough to compete against the PRN which was provoked by the termination of  $200^\circ/\text{sec}$  rotatory stimulus. As a result, compared to the pure PRN, the change in PRN in this situation was found to be minimal. Therefore, a correlation was found between the degree of optokinetic and the degree of PRN inhibition.

## ACKNOWLEDGEMENT

This study was supported in part by NASA Contract NAS 9 14546 NIH grants NS 10940 and NS-07237

## ZUSAMMENFASSUNG

Wenn der Totenkopffläche eine gute Fähigkeit erweist ein Ziel zu verfolgen dann tritt eine Erhöhung oder eine Hemmung des nachreizenden Nystagmus auf.  
die  
kine  
Die  
nystagmus und Nachdrehnystagmus hervorrufen sind nicht gleich. Es ist jedoch eine Ähnlichkeit vorhanden in der Reaktion der Kennzeichen und der Kreuzweisen gegenseitigen Beeinflussung die sehr stark auf das Vorhandensein von gemeinsamen Nervenverbindungen zwischen dem Reizempfänger und dem Reizgeber verweisen.

## REFERENCES

Azzena G B Azzena M T & Marim R 1974 Ac

Blo

Bra

centric and exocentric motion perception *Exp Brain Res* 16 476

Cohen B Uemura T & Takemori S 1973 Effects of labyrinthectomy on optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN) *Equilibrium Res* 3 88

Collins W E 1968 Modification of vestibular nystagmus and vertigo by means of visual stimulation *Trans Am Acad Ophthalmol Otol* 72 962

Collins W E 1968 Special effects of brief periods of visual fixation on nystagmus and sensations of turning *Aerospace Med* 39 257

Dichgans J & Brandt Th 1972 Visual vestibular interaction and motion perception *Bibl Ophthalmol* 82 327

— 1973 Optokinetic motion sickness and pseudo corollis effects induced by moving visual stimuli *Acta Otolaryngol (Stockh)* 76 339

Dichgans J Schmidt C L & Graf W 1973 Visual input improves the speedometer function of the vestibular nuclei in the goldfish *Exp Brain Res* 18 319

Dodge R 1923 Adequacy of reflex compensatory eye movements including the effects of neural rivalry and competition *J Exp Psychol* 6 169

Fischer M H & Kornmüller A E Cited from Dichgans J & Brandt Th 1972

Fukuda T Himoki M & Tokita T 1957 Provocation of labyrinthine reflex by visual stimuli. Evaluation of the theory of subliminal rotation *Acta Otolaryng (Stockh)* 48 425

Fukuda T & Tokita T 1964 Diagonal nystagmus: influence of optokinetic stimulation on postrotatory caloric nystagmus *Confin Neurol* 24 127

Guedry F E Jr, Collins W E & Sheffey P L 1966 Perceptual and oculomotor reactions to interactive visual and vestibular stimulation *Percept Mot Skills* 12 387

Gurnee H 1931 The effect of a visual stimulus upon the perception of bodily motion *Am J Psychol* 41 3

Henn V, Young L R & Finley C 1974 Vestibular nucleus units in alert monkeys are also influenced by

Kli

Kornhuber H H 1962 Optokinetischer Nachnystagmus vestibuläre Übererregbarkeit und periodischer Nystagmus alternans *Ann Psychiatr* 40 549

Krieger H P & Bender M 1956 Optokinetic after nystagmus in the monkey *Electroencephalogr Clin*

Mowrer O H 1935 Some neglected factors which influence the duration of post rotational nystagmus *Acta Otolaryngol (Stockh)* 22 1

— 1937 The influence of vision during bodily rotation

vestibulo-ocular reflex *Proc Fifth Extramural Meeting of the Barany Society (Kyoto)* pp 130-141

Shanzer S, Teny P, Krieger H P & Bender M 1958 Defects in optokinetic after nystagmus in lesions of the brainstem *Am J Physiol* 194 419

Takemori S 1974 The similarities of optokinetic after nystagmus to the vestibular nystagmus *Ann Otol Rhinol Laryngol* 83 230

Young L R, Dichgans J, Murphy R & Brandt Th 1973 Interaction of optokinetic and vestibular stimuli in motion perception *Acta Otolaryngol (Stockh)* 76 24

M Igarashi MD  
Dept of Otorhinolaryngology  
Baylor College of Medicine  
Houston TX 77030 USA

## VISUAL-VESTIBULAR INTERACTION UPON NYSTAGMUS SLOW PHASE VELOCITY IN MAN

E. Koening,<sup>1</sup> J. H. J. Allum<sup>2</sup> and J. Dichgans<sup>1</sup>

*From the Neurological Clinic University of Freiburg Freiburg im Breisgau BRD*

(Received August III 1977)

**act** The influence of vestibular and visual (optokinetic) stimuli on the nystagmus slow phase velocity in man was studied using different combinations of visual horizon and/or passive body rotations (velocity zoids). The interactions of combined stimulation were evaluated in comparison to pure optokinetic and vestibular reactions. The results indicate that retinal image stabilization and vestibular systems simultaneously alter ocular reflexes during passive body accelerations.

• light  
• dark

Exclusive vestibular stimulation by passive body rotation in the dark yields a rather low gain of the vestibulo-ocular reflex (VOR) with respect to acceleration. With optokinetic stimulation the gain depends on stimulus velocity. It is close to unity at velocities below 10°/s and progressively decreases with increasing pattern velocity.

During passive body rotation in the light (a concomitant visual and vestibular stimulation occurs) the response profile suggests that both visual and vestibular inputs contribute to the response. Compared to pure optokinetic stimulation this results in a better correspondence of the SPV and stimulus velocity at low accelerations and velocities.

When subjects viewed a surround rotating with constant relative velocity the amount of enhancement or depression of SPV by the additional application of passive body rotation increased with the body acceleration and with surround velocity. With small surround velocities and high enhancing body accelerations the SPV may be higher than the relative surround velocity. Depressing vestibular stimuli cause a greater SPV modulation than enhancing of the stimuli, sometimes even reversing the direction of nystagmus. The results indicate an interaction between optokinetic (visual) and vestibular inputs.

Visual (retinal image stabilization) and vestibular reflexes may initiate eye movements as the head is moved freely within a normally lit environment. Since it is known that both systems, if separately stimulated under optimal conditions, can work at a gain close to unity

(Barr et al., 1976, Morasso et al., in prep., Dodge et al., 1930, Mackensen, 1954) the question arises as to how the individual gains are adjusted in cases where both channels could be effective at the same time. Interaction between the two reflexes has earlier been demonstrated by a number of authors (Ohm, 1932, Ter Braak, 1936, Jung & Mittermaier, 1939, Jung, 1948, Guttich & Okamoto, 1964) but has never been thoroughly quantified.

In this paper we have attempted to quantify the interaction between vestibular and visual (optokinetic) stimuli by examining nystagmus slow phase velocity (SPV) under quasi natural, and artificial conditions, with combinations of mutually enhancing and depressing stimuli, consisting of passive body rotations and visual surround motions. The results indicate that the signals created by the retinal image stabilization and vestibular reflex systems may be combined in a weighted sum by the central nervous system to produce a single SPV output. This interaction may serve as a basis for the interpretation of clinical data on optokinetic nystagmus SPV in patients with spontaneous nystagmus due to peripheral vestibular lesions. The following paper (Brandt et al., 1978) presents the clinical data.

This work was supported by the Deutsche Forschungsgemeinschaft SFB 70 (Hirnforschung und Sinnesphysiologie).

<sup>1</sup> Presently at Neurological Clinic Liebert University of Tübingen

<sup>2</sup> Presently at Institut für Hirnforschung Zürich



## METHODS

*Subjects*

Four subjects (three male, one female) were selected from a group of 40 students on the basis of their stable slow phase velocity and regular nystagmus frequency in response to constant optokinetic stimulation. They had normal vestibular function and were familiar with our apparatus from previous experiments.

*Apparatus*

For the experiments, subjects were seated in a chair which rotated about the axis of a surrounding cylindrical drum, 1.5 m in diameter, the inner walls of which were covered with 48 alternating black and white vertical stripes each subtending 7.5 degrees of visual angle (Tonniès, Freiburg). The moving pattern filled the entire visual field. Both the chair and the drum could be rotated (independently or coupled) at servo-controlled velocities. Constant accelerations which ranged between 3 and 12°/sec<sup>2</sup> were applied to either chair or drum, or both. The commanded relative displacement between chair and drum was verified by means of a photocell attached to the chair and placed facing the stripe pattern. Horizontal eye movements were recorded (Jung, 1953) using Beckman silver-silver chloride electrodes. After d.c. amplification, the eye movements and velocity signals from drum and chair were stored on FM magnetic tape for later analysis. Calibrations of eye movements were taken from voluntary saccades (four targets, at 15° and 30° left and right). These were repeated several times during each experiment.

*Experimental procedure*

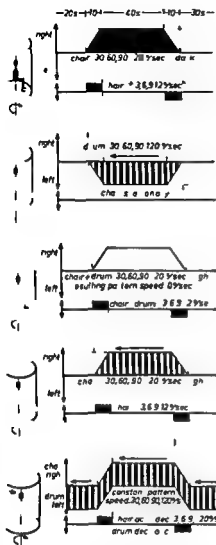
Each subject was seated with his head restrained by a head holder and was asked to attentively pursue the stripe pattern. With pure vestibular stimulation in the dark the eyes were open. The stimuli used are schematically depicted in Fig. 1. They were ve-

locity trapezoids each consisting of an intra-stationary period, a constant acceleration period (3, 6, 9, 12°/sec<sup>2</sup> for 10 sec), a constant velocity period (30, 60, 90, 120°/sec for 40 sec), a constant deceleration period (3, 6, 9, 12°/sec<sup>2</sup> for 10 sec) and a final stationary period. Stimuli consisted either of (a) exclusive chair (body) rotation in the dark (vestibular stimulus), or (b) exclusive drum rotation (optokinetic stimulus). Suppression of vestibular nystagmus by the presence of a stationary pattern (c) was tested by moving the drum and the chair while they were mechanically coupled together ('fixation suppression'). Vestibular and optokinetic stimulation was combined in a natural way (d) by rotating the chair (body) in the light ('natural combination'). The vestibular modulation (e) of optokinetic nystagmus (OKN) in response to constant velocity pattern motion (30, 60, 90, 120°/sec) was studied by the intermittent application of body accelerations and decelerations (3, 6, 9, 12°/sec<sup>2</sup>), which enhanced or depressed the SPV ('interaction trials'). The combination of four accelerations and four pattern velocities resulted in 16 trials for the experiment. After an initial period of pure optokinetic stimulation (drum rotation) lasting 20 sec, subjects were submitted to a 10 sec period of chair acceleration from rest in the opposite direction while the drum was decelerated at the same rate, thus keeping the optokinetic pattern speed—relative to the subject—constant. Thereafter for 40 sec the chair was rotated at constant speed while the relative velocity between drum and chair remained constant. Then a reverse acceleration of chair and drum was applied to reach the original velocity levels. The sequence of the trials was reversed for 2 of the 4 subjects.

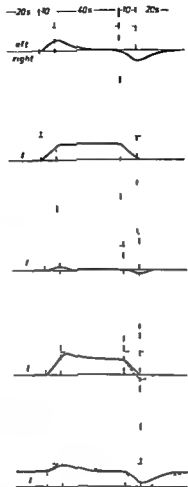
*Data analysis*

The eye movements, recorded on tape, were analog to digital converted every 10 msec, stored on digital computer disks and analysed with a modified Fortran version of the computer program MITNYS II (Allum et al.

## cal realization of stimuli



## Response slow phase velocity of nystagmus



1 Stimulus combinations of experiments a-e and the typical SPV responses (schema)

2) The program automatically computed every 10 msec sample the slow phase velocity of nystagmus and the consecutive 2 sec average of SPV. The program used a linear integration rather than a cubic or sinusoidal integration based on the voluntary saccades initiated between targets at  $0^\circ$ ,  $15^\circ$  and  $30^\circ$  and left. Because of the limited storage of the computer (8K IBM 1130) only 1 sec could be analysed at one time. Thus, ac-

celeration and deceleration periods had to be processed separately. Digital plots presented the original nystagmus together with the slow phase velocity time history. The values of 2 sec SPV averages employed for this paper were taken from the computer printouts. These values were used for the computation of the average maximum and minimum SPV for our 4 subjects. For computations of the average time history of SPV the

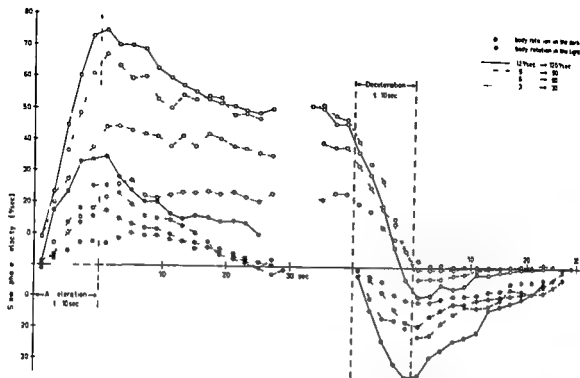


Fig 2 Time course of SPV during pure vestibular stimulation (●) and during natural combination of vestibular

and optokinetic stimulation (○) at different acceleration and corresponding plateau velocities

2 sec average values were linearly interpolated with respect to acceleration or deceleration onset

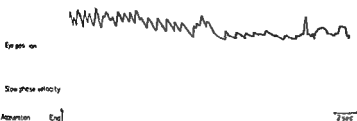
## RESULTS

### Pure vestibular stimulation

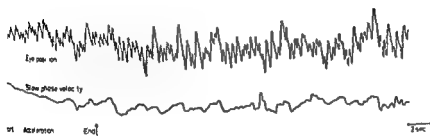
Velocity trapezoids of body rotation in the dark (Fig 1a) caused per and post rotatory vestibular nystagmus, the average time course of which is depicted in Fig 2. Fig 3a shows a representative original recording with the corresponding computer plot. The increase of SPV during the 10 sec acceleration period is well fitted by a straight line (average coefficient of determination ( $r^2$ ) is 0.92, standard deviation (S.D.) = 0.15). With pure vestibular stimulation, the SPV always progressively lagged behind the increasing chair velocity as the vestibulo-ocular reflex (VOR) long time constant became more effective. The vestibular response latency was included in the regression calculations. The accelerations of the

chair (3, 6, 9, 12°/sec<sup>2</sup>) caused an increase in SPV of 1.2, 2.2, 2.9 and 4.0 degrees/sec<sup>2</sup> respectively. The ratios between the corresponding values were employed as a measure of vestibulo-ocular reflex gain (0.41, 0.37, 0.32, 0.34). When the decay effect of the vestibulo-ocular reflex long time constant is accounted for, the true acceleration gain is 35% greater than the value obtained by linear regression techniques. The decay can be observed after the end of acceleration but of course it is effective during the acceleration period too. Maximum SPV (Fig 4) is reached at an average of 1.1 sec after the end of acceleration. This latency of the maximum SPV tended to decrease with increasing accelerations. The decline in SPV during steady chair rotation was fitted by a single exponential function which has an associated average time constant of 15.2 sec (S.D. = 7.2). Deceleration of the chair elicited a nystagmic response in the opposite direction which was symmetrical to the response during the acceleration period.

Pure vestibular stimulation (body rotation in dark)  $99\text{sec}^{-2}$



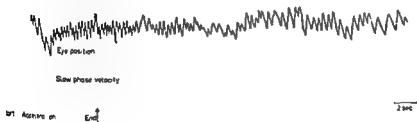
Pure optokinetic stimulation (drum rotation)  $99\text{sec}^{-2}$  -  $90\text{sec}^{-2}$



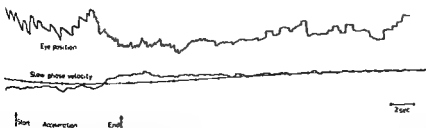
Further suppression (chair acceleration  $99\text{sec}^{-2}$  - pattern velocity  $69\text{sec}^{-2}$ )



Body rotation in light (chair acceleration  $99\text{sec}^{-2}$  chair velocity  $90\text{sec}^{-1}$ )



Body rotation in light (chair velocity  $90\text{sec}^{-1}$  chair deceleration  $99\text{sec}^{-2}$  chair velocity  $69\text{sec}^{-1}$ )



3 Original recordings of nystagmus with the corresponding computer plots from one subject (a) pure vestibular stimulation (b) pure optokinetic stimulation (c) pronounced vestibular nystagmus despite the presence

of a stationary surrounding pattern (d) acceleration (e) deceleration phase during body rotation in the (natural combination) note reversal of nystagmus tubular stimulus)

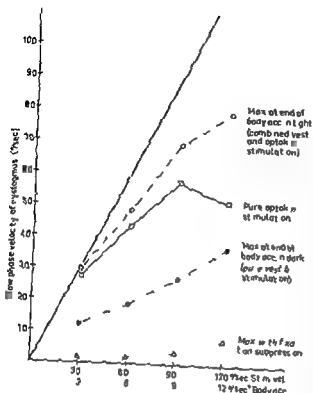


Fig 4 Averages of the four subjects for maximum SPV during acceleration (●) and averages of maximum SPV during the fixation suppression experiment (Δ)

● Max at end of body acc. n light (combined vestib and optokin stimulation)  
○ Pure optokin stimulation  
● Max at end of body acc. n dark (low or vestib stimulation only)  
Δ Max with fixation suppression

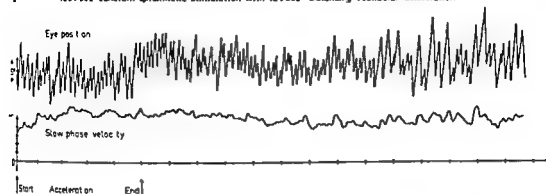
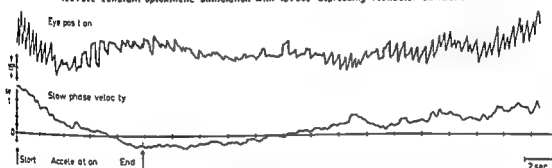
### Pure optokinetic stimulation

With full field optokinetic stimulation elicited by rotating the striped drum around the subject (Fig 1b), SPV closely matched stimulus speed up to 30°/sec, for all accelerations tested except 12°/sec<sup>2</sup>. The gains for the SPV during the acceleration period were 0.78, 0.63, 0.68, 0.67. Periods at the end of the acceleration, where the SPV had saturated for higher pattern velocities, were excluded from the regression calculations. At higher pattern velocities, the SPV was progressively less than the stimulus speed. The maximal steady state SPV was reached at a pattern velocity of approximately 90°/sec and decreased at higher speeds (Fig 4). It showed considerable interindividual variability (range 52–71°/sec for 90°/sec stimulus, among our subjects). The maximum OKN gain (maximum values of SPV divided

by stimulus speed) decreased from speeds of 30°/sec to a value of 0.5 at 120°/sec. The velocity gain (average values 0.9, 0.63, 0.43 for 30, 60, 90, 120°/sec respectively) was maintained throughout the constant velocity period. Maximum SPV of the nystagmus was normally reached after the termination of the acceleration. Some waxing and waning of the SPV occurred during the constant velocity period (Fig 3b). The standard deviation associated with this variability amounted to 6.6, 4.4, 5.8, 7.9% of the individual average SPV, respectively, for the stimulus velocities tested. During the deceleration phase, the SPV remained at the level acquired during constant velocity stimulation and only decreased when the stimulus was close to the level of the SPV. Once the SPV decreased, its gain during the deceleration period was 0.96, 0.82, 0.87, 0.85 (in order of increasing deceleration) thus being greater than the acceleration gains.

### Vestibular stimulation in the presence of a stationary optokinetic pattern (fixation suppression)

When chair and drum mechanically coupled together are accelerated at the same rate (Fig 1c) the use of the retinal stabilization of the striped pattern which is stationary with respect to the chair dramatically decreased the nystagmus response caused by the vestibular stimulus. The effects of suppression lead to a highly variable maximum SPV which amounted on average, to 13% of the maximum SPV during pure vestibular stimulation. The variability of the SPV hampered any assessment of its decay time constants. For the acceleration we used, stable fixation in some cases could not be recovered before or just after the end of the acceleration period but in others nystagmus also persisted up to 20 sec after the end of the acceleration. An original recording of a particularly pronounced nystagmus elicited by body acceleration of 9°/sec<sup>2</sup> in the presence of a stationary pattern is shown in Fig 3c.

120°/sec constant optokinetic stimulation with 12°/sec<sup>2</sup> enhancing vestibular stimulation120°/sec constant optokinetic stimulation with 12°/sec<sup>2</sup> depressing vestibular stimulation

5 Original recording of nystagmus and corresponding computer plots during a 120°/sec constant velocity optokinetic stimulation combined with (a) 12°/sec<sup>2</sup> enhancing

and (b) 12°/sec<sup>2</sup> depressing vestibular stimulation (note the increase in SPV and frequency of nystagmus in (a) and the reversal of nystagmus in (b))

### Natural combination of vestibular and optokinetic stimulation

When velocity trapezoids are applied to subjects seated in a rotating chair while the illuminated drum is kept stationary (Fig 1d) vestibular and optokinetic stimuli are combined in a quasi natural way (Fig 3d). The gains of the SPV during the acceleration period (0.72, 0.64, 0.74, and 0.73) did not differ significantly from those obtained by optokinetic stimulation (excluding saturation effects). The average gain factors with respect to stimulus velocity, as calculated from the maximum SPV (0.99, 0.80, 0.76 and 0.66 for the four velocities tested). Thus increasing vestibular stimulation results in a better transient response for all velocities compared with optokinetic stimulation. Nonetheless, the maximum SPV gain factors were less than one

would have expected from the sum of the corresponding individual gains for optokinetic and pure vestibular stimulation.

The maximum SPV (2 sec average value) as for pure vestibular stimulation, was reached after termination of the acceleration period. The delay between acceleration and the maximum SPV decreased with increasing acceleration (5.6 sec, S.D. 1.6, 3.0 sec, S.D. 1.9, 0.4 sec, S.D. 0.9, 0.3, S.D. 1.0 for the four accelerations 3–12°/sec<sup>2</sup> and the corresponding velocities 30–120°/sec tested). After its peak, the SPV decayed to an average plateau which, 23 sec after the end of acceleration, had a velocity gain of 0.72, 0.68, 0.53, and 0.40. The difference with respect to the optokinetic gain measured at the same time is statistically significant. The time constant of the single exponential fit to the de-

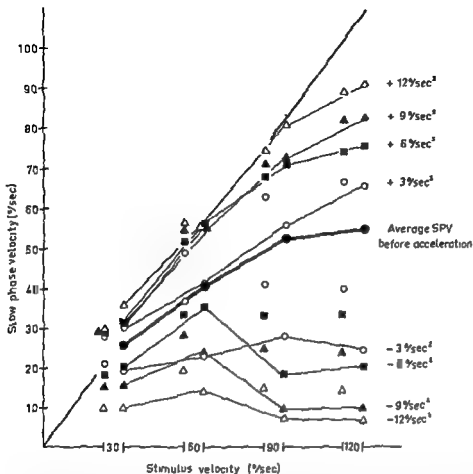


Fig. 6 Comparison of obtained and predicted maximum and minimum SPV values elicited by additional vestibular modulation during constant velocity pattern motion. The obtained with the combination of each pattern velocity and each acceleration is represented by 2 values by identical symbols. The value further on the right hand side for any given stimulus velocity shows the

experimentally obtained SPV. The experimentally obtained values are connected by lines. The value further to the left shows the SPV predicted on the basis of the formula given in the algebraic summation hypothesis. The amount of SPV modulation can be obtained by comparing the maximum and minimum SPV values with the SPV before acceleration onset.

sec, S.D. 7.7) is not significantly less than the time constant obtained for body rotation in the dark.

With the onset of deceleration, the vestibular component of the stimulus immediately depressed the SPV even though the relative motion of the optokinetic pattern, on the basis of the OKN deceleration response, would tend to maintain SPV at the plateau level (Fig. 2). The decrease of the SPV ( $-2.2$ ,  $-3.6$ ,  $-4.8$ ,  $-5.9$   $^{\circ}/\text{sec}^2$ ) during deceleration, however, was more shallow than the increase of the SPV during the acceleration phase ( $2.2$ ,  $3.9$ ,  $6.7$ ,  $8.1$   $^{\circ}/\text{sec}^2$ ). A deceleration of  $3^{\circ}/\text{sec}^2$ , caused

nystagmus to cease once the rotating chair was at rest. With decelerations equal to or greater than  $6^{\circ}/\text{sec}^2$ , the direction of nystagmus frequently reversed (Fig. 3e). This was seen in 4 subjects with a deceleration of  $12^{\circ}/\text{sec}^2$ . The reversal may even start before the end of the deceleration period. The amount of postrotatory nystagmus differed widely for different subjects. Subjects with high SPV during the constant velocity phase showed no or only little nystagmus reversal at the end of the deceleration phase. With slow SPV prior to deceleration subjects tended to show strong postrotatory nystagmus. A persisting inter-

tion between the optokinetic and vestibular system became apparent after the chair had come to rest. During this period the reversed nystagmus decayed to zero velocity with an average time constant of 6.7 sec (S.D. 1.0). This is shorter than the vestibulo-ocular reflex time constant as determined by body rotation in the dark.

#### *Stibular stimulation in the presence of a constant velocity pattern*

In this group of experiments (Fig. 1e), after cancelling optokinetic nystagmus by rotating the speeded drum, subjects were accelerated in the opposite direction. This resulted in an enhancement of the SPV, even though the drum was decelerated at the same rate, thereby keeping constant the relative surround motion and therefore, the optokinetic stimulus speed (see original recordings in Fig. 5a). On reversing the procedure by decelerating the subjects, SPV was depressed (see Fig. 1e). Complete suppression of OKN or even reversal in nystagmus direction was observed in 2 of the subjects with depressing accelerations of 9 and 12°/sec and pattern velocities of 90 and 120°/sec (Fig. 5b). With suitable stimulus conditions (30°/sec pattern velocity and 12°/sec<sup>2</sup> vestibular acceleration), enhancement may lead to a SPV that over several seconds (average 7.0, S.D.  $\pm 4.7$ ) exceeded pattern speed by a few degrees per second. This overshoot reversed the direction of retinal image motion. It is possible for calibration errors in eye movements to cause such small differences. This artifact, however, was excluded as a possible cause when 6 additional subjects observed a retinal after image motion faster than the pattern velocity. Normal optokinetic stimulation yields an after-image motion slower than the stimulus velocity.

Both the rate of change in SPV during the acceleration phase and the maximum SPV increased with vestibular acceleration rate and the optokinetic pattern velocity. Fig. 6 shows the averages for the maximum and minimum SPV for different accelerations and decelerations

combined with different pattern velocities. To evaluate the amount of modulation, the mean SPV before acceleration started is also shown. The figure clearly demonstrates that the extent of modulation increases not only with the body acceleration but also with higher pattern velocities, and that, on the average, depressing vestibular stimuli have greater effects than enhancing ones. The maximum SPV was reached at an average of 1.2 sec after the end of acceleration, however, this delay varied considerably (S.D. 5.1). Occasionally SPV peaked several seconds before the end of acceleration. The minimum SPV for depressing stimuli occurred, on average, 3.4 sec after acceleration had ended.

The single exponential fit of the SPV decay after the SPV maximum during depress and enhance trials has an average time constant of 15.9 sec. For the trials using small accelerations and small pattern velocities, it was not possible to assess the time constant, because of spontaneous fluctuations in the SPV from which a clear acceleration related SPV modulation was difficult to discriminate. Because of the small difference of this time constant compared with that for pure vestibular stimulation we concluded that, at least for high accelerations and high pattern speeds, the time course of the vestibular modulation of OKN is determined by the dynamics of the vestibulo-ocular reflex.

## DISCUSSION

The aim of this study was to determine the interactions of two reflex systems, the optokinetic (retinal image stabilization) system and the vestibulo-ocular reflex (VOR) which converge to produce nystagmus as a common response. The experimental conditions were arranged to activate either reflex alone or both together. The data from the pure vestibular and the pure optokinetic trials served as basis for the evaluation of the trials, with both reflexes interact.

The results of the vestibular stim



passive body rotation in the dark are in agreement with the findings of Brown & Crampton (1964). The gain obtained with trapezoid velocity profiles does not differ significantly from gain values (0.4–0.6) obtained when subjects are rotated sinusoidally in the dark at a frequency of 0.1 Hz (Meiry, 1971, Sugie & Melvill Jones, 1971, Barr et al., 1976). All these studies, including ours, indicate a low gain for the passive VOR at low frequencies. In contrast, the passive VOR gain rises to unity for high frequency rotation of 3 Hz (Benson, 1970) or sharp transients (Barr et al., 1976). Thus the low value of the VOR obtained with passive head movements may not be a functional deficit unless active head rotations have significant components at low frequencies. The dependency of the optokinetic nystagmus gain upon pattern velocity found in the pure optokinetic experiments agrees with earlier findings of Gruttner (1939), Mackensen (1954), Honrubia et al. (1968) and Dichgans et al. (1973). An increasing mismatch between stimulus and eye velocities appears in a similar range for pursuit movements (Dodge et al., 1930). This correspondence between optokinetic and pursuit systems, apparent in the low frequency responses and latencies to changes in stimulus velocity, has been used by Yasui (1974).

### VOR-suppression

Our results, for conditions when the visual surround rotates with the subject, show that the VOR SPV response is not simply cancelled by the presence of a stationary pattern. The irregularity of the response suggests that the degree of suppression is dependent on the attention paid to the stationary contrasts. Our experiments with a stereotyped visual surround, with numerous equal contrasts possibly being fixated on, are not identical with experiments using one small fixation mark. Our vestibular stimulus is not to be compared with repetitive sinusoidal rotations because of their higher predictability. Experiments with sinusoidal stimulation show a much better VOR

suppression that is weakest above 1 Hz when the VOR frequency response increases (Barr et al., 1976, Benson 1970) and the optokinetic frequency response decreases (Fender & Nye 1961, Yasui, 1974).

### Natural combination and interaction trials

In the natural combination experiments and in the interaction trials the VOR and the retinal image stabilization reflex are both activated. Three possibilities of how both reflexes, when active at the same time, could contribute to the nystagmus response have to be taken into account: a switching mechanism, a weighted summation, or an algebraic summation.

**Switching Hypothesis** The switching hypothesis (Waespe & Henn 1977) assumes that either the visual or the vestibular input is causing the SPV, the activity from the other input being suppressed. From the SPV response observed in the natural combination experiment we conclude that a switching cannot be the underlying mechanism. The high gains during the acceleration phase compared with pure vestibular stimulation suggest a contribution of the visual input. Also SPV at the end of body accelerations in the natural combination experiment is faster than that observed for pure optokinetic stimulation. The decline of SPV after the end of acceleration in the natural combination experiments occurs with a time constant similar to the vestibular decay and indicates that the vestibular input contributed to the response. A further argument against the switching hypothesis is given by the vestibularly induced modulation of SPV in the interaction trials, which should be absent if a switching mechanism were active. The observed SPV profiles can be explained on the basis of the two other hypotheses which suggest different concepts of how sensory signals are combined at premotor structures to produce a single SPV output.

**Weighted Summation** During body acceleration in the presence of a stationary visual surround retinal image stabilization reflex

VOR are simultaneously active. With this of combined stimulation the question arises as to the input controlling the retinal image stabilization reflex. In contrast with the ebraic summation hypothesis discussed or the weighted summation hypothesis as that the input controlling the optokinetic contribution of the retinal image stabilization reflex is proportional to surround velocity. Testing this assumption by adding the results obtained during separate optokinetic and vestibular stimulation shows that the effects of the combined stimulation are not purely additive, especially during acceleration when the added gains for separate stimulation are, on the average, 47% greater than the combined stimulation gains. One explanation for the observed effects could be a gain modification of either one or both reflex signals occurs before they are combined i.e. a weighted summation, so that a SPV exceeding the stimulus speed is prevented. A similar non additive result for the SPV has been observed in a non foveate animal the rabbit, using sinusoidal stimulation (Colewyn, 1974).

The scaling down of the vestibular input could be elicited by the presence of retinal image stabilization, whereas the optokinetic input to the vestibular system could be reduced by the presence of body rotation. From the natural combination experiments one may conclude that the reduction of the optokinetic gain induced by the vestibular system is relatively minor. In these experiments the SPV at constant velocity body rotation progressively decreases with ceasing vestibular excitation to equal the SPV during pure optokinetic stimulation.

On examining the results for interaction trials (Fig. 6) an idea can be obtained of how the vestibular weighting factors may change with stimulus magnitude. As relative stripe velocity is increased the vestibular weighting factor for the same vestibular stimulation would increase i.e. the optokinetic input would influence the vestibular gain weighting.

The advantage of the increase in the weight

ing factors with stimulus magnitude is apparent from the body rotation in the light trials. With separate stimulation the gain of each reflex tends to decrease with stimulus acceleration. If, however, the weighting factor of each reflex in the weighted summation on combined stimulation were to increase with stimulus magnitude, the effect of gain decrease on the separate reflexes would be minimized and a more linear response result. In fact, acceleration gains in the natural combination experiments are constant throughout the accelerations tested.

The question arises as to whether retinal error velocity alone is the controlling input to the optokinetic component of the SPV during natural stimulation and the interaction trials. Two of our results indicate that this is probably not the case. Firstly, the enhancement and depression of SPV occurs for periods of several seconds during the interaction trials. Secondly, the time constant associated with the decay of SPV during the natural stimulation constant velocity period is close to that corresponding to pure vestibular stimulation. A priori, one would have expected the retinal image stabilization reflex, from its known response latency to error velocity (Rashbass, 1961, Robinson, 1965), to have compensated for the retinal error after its latency (100–200 msec) in these two experiments. These results, however, can be explained if one assumes that surround velocity is represented in the central nervous system and is controlling the optokinetic contribution to the SPV. This representation would consist of a positive feedback of eye velocity added to the retinal error velocity signal. In this case one should assume that the steady state gain of the eye velocity signal and the retinal error velocity signal when summed are less than unity, thus explaining the reduced pursuit tracking ability above 25 to 30°/sec (Young, 1971).

With the representation of surround velocity in the central nervous system as an input to the SPV, the mechanism of depression of SPV for the

be explained. For the enhancement trials the vestibular signal together with the positive feedback of eye velocity seems to override any correcting influence of the retinal error velocity signal. The overshoot of eye velocity continued for as long as the vestibular signal reinforced by positive feedback exists. The enhancement is greater with increasing optokinetic stimulation magnitudes because of the increase in the weighting factors of the vestibular reflex contributing to the total SPV response. Similarly for the depression of SPV by the vestibular stimulation the effect of positive eye velocity feedback maintains the depression. The eye velocity signal and the vestibular signal are greater than the correcting signal proportional to retinal error velocity.

**Algebraic Summation** At the onset of the natural combination experiments the vestibularly induced SPV is thought to decrease retinal slip and thereby the optokinetic input after a short latency of a few milliseconds, before eye movements on the basis of the optokinetic stimulation have been elicited (latency 100–200 msec, Robinson, 1965). This leads to the assumption that the optokinetic input might be the difference between surround velocity and a vestibularly induced SPV component. The vestibularly induced SPV component is assumed to be identical with the SPV response to pure vestibular stimulation in the dark by the respective chair acceleration. On the basis of these assumptions the SPV during the combined vestibular and optokinetic trials was predicted from the SPV observed during separate optokinetic and vestibular stimulation using the following formula

$D$  = drum velocity

$C$  = chair velocity

$D - C$  = optokinetic surround velocity i.e. drum vel - chair vel

$g_v$  = gain during pure vestibular stimulation

$g_v C$  = SPV during pure vestibular stimulation = vestibular component

$(D - C) - g_v C$  = effective optokinetic input i.e. stimulus velocity minus VOR induced SPV

$g_o$  = velocity dependent gain as determined during pure optokinetic stimulation

$g_o((D - C) - g_v C)$  = OKN component of SPV expected from the effective optokinetic input on the basis of the velocity dependent gain observed during pure optokinetic stimulation

$g_o((D - C) - g_v C) + g_v C$  = predicted SPV = summation of the optokinetic and the vestibular component

For the trials with vestibular decelerations signs for the vestibular component had to be reversed.

The predicted maximum SPV (average for our 4 subjects) in the natural combination experiments are 28.4, 51.8, 71.2 and 89.4°/sec for the four accelerations and velocities. To compare them with the observed maximum values (29.5, 48.5, 68.6, 78.7) we used the ratios (observed value/predicted value) which are 1.093, 0.96, 0.88.

Although the optokinetic stimulation preceded the vestibular stimulation in the 'interaction trials' the same method of predicting the maximum SPV from separate vestibular and optokinetic stimulation was used because the retinal image motion was also changed in these experiments by the vestibular stimulation. Fig. 6 compares the observed SPV values with the expected maxima. For enhancing stimulation the observed values are on the average 6°/sec higher than the expected values.

Of particular interest is the finding of a SPV faster than the stimulus when a 12°/sec<sup>2</sup> vestibular acceleration was combined with a 1 sec pattern motion. A priori one would have assumed that the reversed retinal image motion, elicited by the SPV faster than the pattern motion, should have decreased the SPV via the optokinetic input after a latency of about 100–200 msec. The reason for the observed overshoot of SPV over the sum of the two components could be long lasting effects of the preceding optokinetic stimulation.

t summed with the VOR. Such long lasting effects have been demonstrated by Ohm (1971), Mackensen et al (1961), Brandt et al (1974), Cohen et al (1977) as optokinetic nystagmus (OKAN). A tonic modulation of the vestibular neurons discharge rate that lasts the visual stimulus may be the neurophysiological basis (Dichgans & Brandt, 1972; Ohm et al 1973; Henn et al 1974; Allum et al 1977) for these phenomena.

In trials with depressing vestibular stimulation the hypothesis assumes that the vestibular stimulus elicits a hypothetical SPV opposite to an optokinetic SPV. Thereby the difference between the vestibular SPV and the optokinetic pattern velocity becomes greater than the actual pattern velocity. Thus the OKN is decreased because of its velocity dependence. Nevertheless the observed minimum SPV values are on the average  $7.2^\circ/\text{sec}$  lower than the predicted SPV (Fig. 6). A possible explanation could be that a storage mechanism in the brain stem, the activity of which gives rise to optokinetic after-nystagmus, is necessary for a high SPV of OKN with high pattern velocities (Cohn et al 1973), is discharged by the vestibular stimulus to the opposite side. The indirect effect of the discharge of this OKAN storage mechanism on OKN-SPV, combined with the direct effect of the vestibular stimulus to the opposite side, could account for the strong inhibition we observed.

Our experiments may be the basis for the understanding of clinical data on OKN abnormalities in patients with vestibular lesions. These data will be discussed in the subsequent paper by Brandt et al (1977).

## ACKNOWLEDGEMENT

We thank Mrs U. Rommelt for Assistance with graphics.

## ZUSAMMENFASSUNG

Der Einfluss vestibularer Beschleunigungsreize auf die Geschwindigkeit der langsamen Phase (SPV) des optokinetischen Nystagmus (OKN) wurde mit verschiedenen

Kombinationen von Umwelt- und passiven Körperrotationen untersucht. Zum Vergleich dienten rein vestibulärer und rein optokinetischer Nystagmus.

### Ergebnisse

1) Während rein vestibulärer Reizung im Dunkeln ergab sich ein Verstärkungsfaktor 0.3–0.4 für den vestibulookularen Reflex im Verhältnis zur Beschleunigung.

2) Während rein optokinetischer Reizung hängt der Verstärkungsfaktor von der Reizmustergerwindigkeit ab. Bis zu Geschwindigkeiten von  $30^\circ/\text{sec}$  ist er nahezu 1 und nimmt dann mit zunehmender Reizmustergerwindigkeit ab.

3) Wurde die Versuchsperson im Hellen bei stationärem Horizont beschleunigt (natürliche Kombination vestibulärer und optokinetischer Reize), so führte die zusätzliche vestibuläre Reizung bei hohen Reizmustergerwindigkeiten im Vergleich zu der unter 2) beschriebenen Versuchsanordnung zu einer schnelleren SPV und damit zu einer besseren Übereinstimmung zwischen SPV und Mustergerwindigkeit.

4) Bei konstanter optokinetischer Reizmustergerwindigkeit nahm die SPV Modulation durch intermittierende zusätzliche vestibuläre Reize mit der Stärke der Beschleunigung und für zunehmende Reizmustergerwindigkeiten zu. Bei niedrigen Reizmustergerwindigkeiten führten starke Beschleunigungen dazu, dass die SPV für mehrere Sekunden die Reizmustergerwindigkeit überstieg. Den OKN hemmende vestibuläre Reize verursachten eine stärkere Modulation der SPV als aktivierende Reize. Hemmende Reize konnten den OKN unterdrücken und vorübergehend einen vestibulären Nystagmus in Gegenrichtung auslösen. Die Ergebnisse lassen auf eine Interaktion von optokinetischen (visuellen) und vestibulären Einflüssen schließen.

## REFERENCES

- Allum J H J, Graf W, Dichgans J & Schmidt C L 1977 Visual vestibular interactions in the vestibular nuclei of the goldfish. *Exp Brain Res* 26: 463.
- Allum J H J, Tole R & Weiss A D 1975 MITNYS: a digital program for on-line analysis of nystagmus. *IEEE Trans Biomed Eng* 22: 773–777.
- Barr C C, Schultheiss L W & Robinson D A 1976 Voluntary non visual control of the human vestibular ocular reflex. *Acta Otolaryngol* (Stockh) 81: 365.
- Benson A J 1970 Interactions between semicircular canals and graviceptors. In *Recent Advances in Aerospace Medicine* (ed D E Busby) D Reidel Dordrecht.
- Brandt Th, Allum J H J & Dichgans J 1978 Computer analysis of optokinetic nystagmus in patients with spontaneous nystagmus of peripheral origin. *Acta Otolaryngol* (Stockh) 85: 5–6.
- Brandt Th, Dichgans J & Buchele W 1977 Habituation. Inverted self motion? optokinetic after-nystagmus. *Exp*

- Brown J H & Crampton G H 1964 Quantification of the human nystagmic response to angular acceleration. Prediction formulae and nomograph *Acta Otolaryngol* (Stockh) 58 555
- Cohen B, Matsuo V & Raphan T 1977 Quantitative analysis of the velocity characteristics of optokinetic nystagmus and optokinetic afternystagmus *J Physiol* (Lond) 2 1
- Cohen B, Uemura T & Takemori S 1973 Effects of labyrinthectomy on optokinetic nystagmus (OKN) and optokinetic afternystagmus (OKAN) *Equilibrium Res* 3 88
- Dichgans J & Brandt Th 1972 Visual vestibular interaction and motion perception. *Cerebral Control of Eye Movements and Motion Perception* (ed J Dichgans & E Bizzi) Bibl Ophthalmol Vol 82 p 327 S. Karger Basel New York
- Dichgans J, Nauck H & Wolpert E 1973 The influence of attention, vigilance and stimulus area on optokinetic and vestibular nystagmus and voluntary saccades. In *The Oculomotor System and Brain Functions* (ed V Ziskmund) Butterworths London
- Dichgans J, Schmidt C L & Graef W 1973 Visual input improves the speedometer functions of the vestibular nuclei in goldfish. *Exp Brain Res* 18 319
- Dodge R, Travis R C & Fox J C 1930 Optic nystagmus. III Characteristics of the slow phase *Arch Neurol Psychol* (Chic) 24 21
- Fender D H & Nye J W 1961 An investigation of the mechanisms of eye movement control *Kybernetik* 1 81
- Gruttner R 1939 Experimentelle Untersuchungen über den optokinetischen Nystagmus *Z Sinnesphysiol* 68 1
- Guttich H & Okamoto K 1964 *Psychophysik* 1 1
- oculus units in alert monkeys are also influenced by moving visual fields *Brain Res* 71 144
- Honrubia V, Dooney D P, Mitchell H A & Ward P H 1968 Experimental studies on optokinetic nystagmus. II Normal humans *Acta Otolaryngol* (Stockh) 65 441
- Jung R 1948 Die Registrierung des postrotatorischen und optokinetischen Nystagmus und die optisch vestibuläre Integration beim Menschen *Acta Otolaryngol* (Stockh) 36 199
- 1953 Nystagmographie. Zur Physiologie und Pathologie des optisch vestibulären Systems beim Menschen *Acta Otolaryngol* (Stockh) 36 199
- Nystagmus in ihren Wechselbeziehungen *Arch Otolaryngol* (Stockh) 146 410
- Mackensen G 1954 Untersuchungen zur Physiologie des optokinetischen Nystagmus *Graefes Arch Ophthalmol* 155 284
- Mackensen G, Kommerell G & Silberssen D 1955 Untersuchungen zur Physiologie des optokinetischen Nachnystagmus. II Mittlung. Individuelle Unterschiede des Nachnystagmus und die Abhängigkeit vom optokinetischen Nachnystagmus von der Reizdauer *Graefes Arch Ophthalmol* 163 170
- Meiry J L 1971 Vestibular and proprioceptive stabilization of eye movements. In *The Control of Eye Movements* (ed P Bach, Y R, C A Collins & J E Hyde) p 483 Academic Press New York London
- Morasso P, Sandini G, Tagliasco V & Zaccaria 1971 Eye-head coordination. Role of the vestibulo-ocular reflex *Exp Brain Res* 234
- Ohm J 1921 Über optischen Drehnystagmus *Monatshbl Augenheilkd* 18 234
- 1932 Über die Beziehungen zwischen visuellen, optischen und vestibulären Augenbewegungen *Hals Nasen Ohrenheilkd* 32 234
- Rashbass C 1961 The relationship between saccadic and smooth tracking eye movements *J Physiol* (Lond) 159 326
- Robinson D A 1965 The mechanics of human smooth eye movements *J Neurophysiol* 28 1
- on Systems Man and Cybernetics SMC 1 101
- Ter Braak J W 1936 Untersuchungen über den optokinetischen Nystagmus *Arch Neurol Physiol* 21 1
- Waespe W & Henn V 1977 Neuronal activity in vestibular nuclei of the alert monkey during vestibular and optokinetic stimulation *Exp Brain Res* 27 59
- Yassin S 1974 Nystagmus generation, oculomotor control and visual motion perception. PhD thesis, MIT
- Young L R 1971 Pursuit eye tracking movement. *The Control of Eye Movements* (ed P Bach, Y R, C A Collins & J E Hyde) p 479 Academic Press New York London

E Koenig M D  
Neurological Clinic  
University of Tübingen  
Liebermeisterstr 18  
74 Tübingen FRG

# QUANTIFICATION OF VESTIBULAR COMPENSATION IN UNILATERAL MENIERE'S DISEASE

M. Stefanelli,<sup>1</sup> E. Mira,<sup>2</sup> R. Schmid<sup>1</sup> and R. Lombardi<sup>1</sup>

From the <sup>1</sup>Institute of Electronics and the <sup>2</sup>Otorhinolaryngological Clinic  
University of Pavia Pavia Italy

(Received June 2 1977)

**Abstract** The main parameters of the vestibulo-ocular (i.e. the gain or reflectivity, the long time constant of the semicircular canals, the time constant of the neural transduction and that of the central integrator) were computed from the post rotational tagmic responses of unilateral Meniere's patients. Comparison with normal subjects showed a decrease of the first two parameters both in CW and CCW responses. Significant changes were observed in the remaining parameters. Probably due to a process of central compensation, a high level of symmetry was observed mainly in the patients.

In recent years, vestibular induced nystagmus has been used to identify the parameters of mathematical models of the vestibulo-ocular (Schmid et al., 1971; Mira et al., 1975a; Schmid, 1975). In particular, the gain or reflectivity, the long time constant of the semicircular canals, the time constant of the chano-neural transduction, and that of the leaky integrator were estimated with sufficient accuracy to discriminate between normal and pathological situations (Mira et al., 1975b).

Nevertheless, this approach has not yet been used to examine a specific pathology in a systematic way. Meniere's disease seems to be a good experimental model for this purpose. Actually, it is generally accepted that Meniere's disease is caused by a distention of the membranous labyrinth produced by an endolymphatic hydrops (Hallpike & Cairns, 1938; Altmann & Kornfeld, 1965; Gussen, 1973). Therefore, the parameters of

tagmic response related to the dynamics of the cupula-endolymph system are likely to vary in Meniere's patients vis a vis normal subjects. On the other hand, the critical imbalance between the labyrinths due to unilateral Meniere's disease may induce a process of central compensation. Thus, in longstanding Meniere's patients, parameters other than peripheral may result varied.

The objective of this work was to verify these predictions by using a model approach to quantify the changes in the nystagmic response of Meniere's patients vis a vis a normal population.

Rotatory tests (post rotational) were adopted. Actually, in this case, the relationships among stimulus, cupula deflection and nystagmus can be precisely defined in mathematical terms (Jones & Milsum, 1965; Sugie & Melville Jones, 1971; Schmid et al., 1971, 1975; Barnes & Benson, 1973; Schmid & Lardini, 1976). By contrast, some uncertainty exists on the relationships between caloric stimulations and cupula deflections. The same caloric stimulus may produce varying effects on the cupula-endolymph system according to the thermal characteristics of the non-homogeneous ear structures through which heat flows.

This work was supported by C.N.R. Special Biomedical Engineering Subproject SFNS.1

FQ Fb 74

Ménière's disease right side  
Post-rotational tests

120°/sec CW



120°/sec CCW



Fig 1 Clockwise (CW) and counterclockwise (CCW) post rotational nystagmus of a patient suffering from a 3-year old Ménière's disease

The mathematical models so far proposed (Steer, 1967, Baertschi et al., 1975, Bock & Bromm, 1977) are the results of heavy simplifications. Moreover, in a recent theoretical and experimental determination of vestibular dynamics in caloric stimulations, Baertschi et al. (1975) concluded that the long-time constant of the semicircular canals cannot be determined from a caloric test since it is masked by dynamics of heat transmission.

Rotatory tests have been considered of limited value in diagnosing Ménière's disease since they give no hint as to which labyrinth is diseased (Stahle, 1968). Caloric tests have been preferred. However, it should be noted that the side of the lesion can be equally or even better recognized on the basis of the clinical history of the patient or from audiometric findings (Hedgecock, 1968). From a clinical point of view, it is probably more interesting to test the degree of dynamic balance of the vestibulo-ocular reflex response acquired by the patient in the everyday life, following processes of central compensation. To this aim, caloric tests are completely inadequate.

## MATERIAL AND METHODS

Two groups of 25 normal subjects and 15 patients with unilateral Ménière's disease were

examined. Both groups were submitted on the same day to a clockwise (CW) and a counter-clockwise (CCW) post-rotational test.

All Ménière's patients had a typical history of disease, with a progressive hearing impairment, tinnitus and attacks of vertigo with nausea and vomiting. Audiological examination showed in all cases a unilateral perceptible hearing loss of various degree, with flat or ascending curve and recruitment measured by the method of Fowler and/or Metz. The duration of the symptoms ranged from 1 to 15 years. Examination of Ménière's patients was performed in vertiginous free intervals and special care was taken to ensure that no signs of labyrinthine imbalance were present and no pharmacological treatment was under taken.

Horizontal nystagmus was recorded in total darkness by means of two bitemporal silver-silver chloride skin electrodes (Beckman Type 3503) with a ground electrode placed on the forehead. The electrodes were connected to a d.c. amplifier (Beckman Dynograph Type R), and chair velocity and nystagmus were displayed simultaneously on a polygraph. The output of the amplifier was also coupled to a four-channel FM tape recorder (Ampex PR 500). After A/D conversion, ENG data were processed by means of an interactive program.

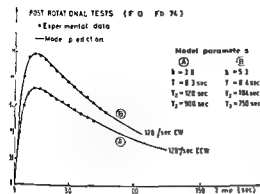


Fig. 2. Diagrams of the slow cumulative eye position (CEP) obtained from the responses in Fig. 1. The values of the model parameters computed through a best fitting procedure are given in the upper right corner.  $K$ : gain of the vestibulo-ocular reflex;  $T_1$ : long time constant of the macular canals;  $T_2$ : time constant of the mechanoneural transduction;  $T_3$ : time constant of the central neural leaky integrator.

Anzaldi & Mira, 1975; Anzaldi et al., 1977) and the diagrams of the slow phase cumulative eye position (SCEP) were constructed.

These diagrams were then fitted by using the following transfer function between the Laplace transform of SCEP and head velocity (Mira et al., 1975)

$$\frac{\text{CEP}}{\Omega} = \frac{KT_1T_2s^2}{(1+sT_1)(1+sT_2)(1+sT_3)} \quad (1)$$

where

$K$  = gain of the VOR,

$T_1$  = long time constant of the lateral semicircular canals,

$T_2$  = time constant of the mechanoneural transduction,

$T_3$  = time constant of the central neural leaky integrator,

$s$  = Laplace variable

The parameters of the transfer function (1) were estimated by using Marquardt's algorithm (1963) as described by Lardani et al. (1975).

Automatic data processing was performed by a general purpose minicomputer (LABEN

70 with 16 k, 16-bit words and 1.35  $\mu\text{sec}$  cycle time) equipped for the graphic presentation of the output (display terminal Tektronix 4010).

In spite of the special care taken in recording and processing nystagmus, the responses of 5 Meniere's cases were so confused by artifacts that they had to be discarded.

All the examined patients were also submitted to caloric tests. Their nystagmic responses were recorded but not processed by computer.

## RESULTS

An example of CW and CCW post rotational nystagmus of a patient suffering from a 3 year old Meniere's disease is given in Fig. 1. The symmetry of the two responses denotes a high degree of compensation. With respect to normal subjects, there is a marked reduction of the response in terms of both beat amplitude and nystagmus duration. These observations are quantitatively confirmed by the values of the model parameters computed from the diagram of the SCEP shown in Fig. 2. Actually, the gain  $K$  computed from the CCW response, corresponding to utriculopetal cupula deflection in the diseased semicircular canal, is about the 70% of the gain computed from the CW response. Both the gains are reduced by about 50% with respect to mean values in normal subjects. No appreciable difference exists between the value of the time constant  $T_1$  computed from the two responses. In this case too a reduction of more than 50% is observed with respect to normal subjects (Schmid et al., 1971; Malcolm, 1973). This finding is in good agreement with the short duration of the primary phase of the response in Fig. 1. The remaining parameters  $T_2$  and  $T_3$ , differ by about 15% when computed from the two responses. Due to the lack of accuracy attending the estimation of these parameters, in particular  $T_3$  (see Appendix), the reported difference cannot be considered as significant. However, the computed values of  $T_2$  are within the range of normality.



Table I

Patient	Age (years)	Duration of disease (years)	Mean hearing loss in dB*	Diseased side	Amplitude (°/sec) and direction of the post rotational test	Parameters of the ENG response		
						number of beats of the 1st phase	duration of the 1st phase (sec)	peak velocity (°/sec)
B U	42	2	R 10 L 75	L	120 CCW 120 CW	33 40	15 16	34 18
D V	38	1	R 38 L 28	R	120 CCW 120 CW	45 60	30 35	90 111
F A	33	3	R 57 L 6	R	90 CCW 90 CW	19 58	20 30	47 33
F Q	58	3	R 52 L 14	R	120 CCW 120 CW	38 35	22 21	47 70
M G	67	2	R 70 L 33	R	150 CCW 150 CW	86 83	33 25	60 100
M A	41	4	R 10 L 42	L	120 CCW 120 CW	60 47	30 28	70 19
L C	34	2	R 72 L 17	R	120 CCW 120 CW	63 53	34 40	20 24
P A	56	2	R 27 L 66	L	120 CCW 120 CW	77 43	45 37	75 37
S C	60	15	R 96 L 57	R	120 CCW 120 CW	44 34	28 20	35 63
V C	56	4	R 51 L 28	R	120 CCW 120 CW	69 42	41 31	75 75

\* Computed at the frequencies of 250 500 1000 2000 and 4000 Hz

Table II

Patient	Diseased side	Amplitude (°/sec) and direction of the post rotational test	SCEP Parameters				
			K	T <sub>1</sub> (sec)	T <sub>2</sub> (sec)	T <sub>3</sub> (sec)	K/T <sub>1</sub>
B U	L	120 CCW	2.3	8.6	75	>1000	0.266
		120 CW	1.7	11.9	54	>1000	0.147
D V	R	120 CCW	8.8	11.5	213	530	0.798
		120 CW	8.3	12.5	308	750	0.666
F A	R	90 CCW	5.2	10.0	180	480	0.422
		90 CW	6.2	16.2	121	183	0.377
F Q	R	120 CCW	3.8	8.3	120	960	0.458
		120 CW	5.3	8.4	104	750	0.633
M G	R	150 CCW	5.1	14.5	200	595	0.353
		150 CW	5.9	9.2	250	950	0.640
M A	L	120 CCW	2.1	14.2	134	800	0.140
		120 CW	1.4	10.0	180	900	0.142
L C	R	120 CCW	3.7	27.3	56	>1000	0.133
		120 CW	3.6	19.7	146	>1000	0.181
P A	L	120 CCW	15.9	25.4	187	>1000	0.625
		120 CW	6.9	25.4	81	770	0.275
S C	R	120 CCW	2.3	8.2	296	940	0.283
		120 CW	2.8	5.1	120	900	0.540
V C	R	120 CCW	4.7	20.4	358	>1000	0.233
		120 CW	8.3	13.2	112	>1000	0.625

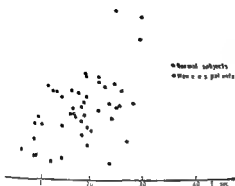


Fig. 3. Values of the gain  $A$  and the long time constant of the semicircular canals for normal subjects (○) and Meniere's patients (●).

Even when all subjects were examined during periods free from vertiginous attacks, a great variability was observed in their nystagmic responses. According to Stahle's observations (1968), spontaneous or positional nystagmus was present in few cases. Its direction was toward as well as away from the diseased ear.

The results obtained from 10 Meniere's patients are summarized in Tables I and II. Table I reports clinical data, the amplitude and direction of the post-rotational stimulation, and the values of some nystagmus parameters (number of beats, duration of the first phase, and peak slow phase velocity). Table II gives the estimated value of the model parameters  $T_1$ ,  $T_2$ , and the ratio  $K/T_1$ . The most outstanding result emerging from Table II is the low values of the parameters  $K$  and  $T_1$ . Fig. 3 shows the values of  $K$  and  $T_1$  for normal subjects and Meniere's patients; the separation between the two sets of data is evident.

As for the balance between CW and CCW responses in Meniere's patients, a comparative analysis suggests that  $K$  is smaller in responses recorded after turning toward the intact side, while no systematic difference exists in  $T_1$ . This observation is supported by the presentation of data as in Fig. 4, where the ratio  $K_D/K_I$  and  $T_{1D}/T_{1I}$  are reported. Sub-

$D$  and  $I$  refer to responses induced by utriculopetal cupula deflections in the diseased and the intact semicircular canal, respectively (that is, post-rotational responses obtained after turning toward the intact and the diseased side). If the two responses were perfectly balanced with no predominance in one particular direction, all the data would fall into a small region centred around the point (1, 1). Since the majority of data are clustered into the lower quadrants, without any significant difference between the right and the left one, it can be concluded that an asymmetry exists only in the sense previously outlined.

When the ratio  $K/T_1$  is computed from CW and CCW responses (see Table II), smaller values are obtained for the diseased side. Simple manipulation of eq. (1) proves that the ratio  $K/T_1$  is proportional to the predicted peak slow phase velocity  $V_p$  of post-rotational responses ( $V_p = AK/T_1$ , where  $A$  is chair velocity before stop). Thus the ratio  $K/T_1$  provides the same information as the maximum intensity defined by Stahle (1968) for caloric tests, and it is equally sensitive to variations in labyrinthine excitability.

The ratio  $K_D/T_{1D}$  is related to the hearing loss in the diseased ear. The results are given in Fig. 5; they prove a strict correlation be-

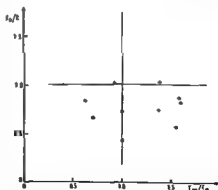


Fig. 4. Balance between the diseased and intact side in terms of  $K$  and  $T_1$ . Subscripts  $D$  and  $I$  refer to responses induced by utriculopetal cupula deflections in the diseased and the intact semicircular canal, respectively (that is, post-rotational responses obtained after turning

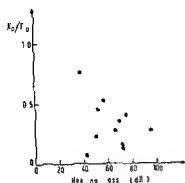


Fig. 5 Relationship between the ratio  $K/T_1$  and the hearing level. The reported the fre

tween these two factors in the sense previously reported by Enander & Stahle (1967) for maximum intensity and mean auditory threshold.

In all the examined patients caloric responses proved an hypoexcitability of the diseased labyrinth and an almost normal excitability of the intact one.

# DISCUSSION

While caloric tests point out a marked asymmetry in the nystagmic response of unilateral Meniere's patients, the same feature does not appear so clearly in post rotational tests. The results reported in this paper show that the parameters  $K$  and  $T_1$  are only slightly different in CW and CCW responses. On the other hand, they are both significantly reduced with respect to normal values. The results are in good agreement with previous findings by Wilmot (1974).

Since the time constant of the intact semicircular canal cannot be varied, its apparent decrease can only be explained as the effect of a process of central compensation taking place through a reduction of the intact side function.

Similar processes have been observed in vestibular compensation following hemilabyrinthectomy in the cat (Precht, 1974; Courjon

et al., 1977). In the acute stage, post rotational stimulations of the injured side do not produce a reversal of spontaneous nystagmus. In the compensated stage, spontaneous nystagmus has disappeared, and a high level of symmetry is attained. Nevertheless, both CW and CCW responses are reduced with respect to normal ones.

The results of electrophysiological investigations suggest that this functional recovery occurs through the following distinct mechanisms: an inhibitory control exerted by vestibulo-cerebellum upon the vestibular nuclei of the intact side, a recalibration of the gains of the commissural pathways between the two sets of vestibular nuclei, and a generation of resting activity in the deafferented vestibular nuclei (Precht et al., 1966; McCabe et al., 1972).

Such a process has been modelled by assuming a two-level hierarchical organization of the vestibulo-ocular system (Jeannerod & Schmid, 1977). The first level was represented by the vestibulo-ocular reflex, while the second one was localized in the cerebellum. When a strong imbalance between the two vestibular receptors occurs, the second level sets up a strategy of control of the lower level aimed

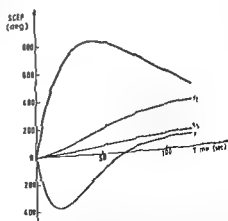


Fig. 6 Upper diagram: time course of the slow eye position (SCEP) for a step change in head velocity. Lower diagrams: time course of the sensitivity coefficients  $v_1$ ,  $v_2$  and  $v_3$  of the SCEP with respect to the time constants  $T_1$ ,  $T_2$  and  $T_3$ .

the re-establishment of the static (absence of spontaneous nystagmus) and dynamic balance symmetry of the responses)

The results reported in this paper suggest that a similar strategy takes place in unilateral Meniere's disease. Since the second level operates through inhibition of the first level, the symmetry of the post-rotational responses can be achieved in Meniere's patients only through a depression of the intact side. Thus, at the compensated stage, both CW and CCW responses of Meniere's patients are reduced with respect to normal subjects. The lower symmetry observed in caloric responses with respect to post-rotational responses can also be explained. In fact, in the former case, due to the unilateral nature of the caloric stimulus, the contribution to the system balance given by recalibration of the gain of the contralateral pathways is lost.

Surprisingly, the data given in Table II indicate no systematic variations of the mechano-neural transduction time constant  $T_2$  between the intact and the diseased side. Moreover, in spite of the great variability of  $T_2$  in Meniere's patients, the observed values can still be considered within the range of normality. This result suggests that at the time of examination, no pathological changes of the labyrinthine epithelium such as to produce appreciable variations in the dynamics of the mechano-neural transduction process were present. It is possible that only the gain of this process was attained. The reduction of the gain of the overall reflex seems to support this hypothesis.

## CONCLUSION

The results reported in this paper seem to re-evaluate the rotatory tests in the examination of Meniere's patients. Mainly for what concerns the evaluation of the dynamic balance between the two vestibular pathways, rotatory tests should be preferred to caloric tests. Actually, vestibular stimulations in everyday life are always bilateral. Thus, it is likely that the fine

control mechanisms developed to maintain or reestablish the balance between the right and the left vestibular pathways are not recruited in the case of unphysiological unilateral stimulation. The persistent asymmetry of the caloric response observed also in longstanding Meniere's patients seems to support this hypothesis. On the contrary, the comparison of the values of the vestibulo-ocular reflex parameters estimated from CW and CCW post-rotational responses indicate the possibility of quantifying the level of compensation. It is also proved that this central compensation is obtained through a depression of the vestibular function of the intact side.

## APPENDIX

The sensitivity of dynamic systems to parameter variations is currently considered in control theory by introducing the *sensitivity coefficients*  $s_i$  defined as

$$s_i(t) = \frac{\delta y(t)}{\delta \ln p_i}$$

where  $y(t)$  is the system output and  $p_i$  is the  $i$ th system parameter (Tomovic 1963). Sensitivity theory can also be used to evaluate the reliability of a parameter estimation from recorded responses. Actually, the greater the sensitivity coefficient of one parameter is, the more accurate its identification results. From this point of view, it is of interest to estimate the sensitivity of the slow cumulative eye position (SCEP) to variations of the parameters  $T_1$ ,  $T_2$  and  $T_3$  characterizing the dynamics of the vestibulo-ocular reflex. We can thus introduce the following sensitivity coefficients

$$s_1(t) = \frac{\delta c(t)}{\delta \ln T_1}, \quad s_2(t) = \frac{\delta c(t)}{\delta \ln T_2}, \quad \text{and} \quad s_3(t) = \frac{\delta c(t)}{\delta \ln T_3}$$

where  $c(t)$  is the time function representing the SCEP.

Fig. 6 shows the diagram of the SCEP predicted by eq. (1) for a step change in the angular velocity. The following mean val

timated on a population of 25 normal subjects were given to model parameters:  $K=10$ ,  $T_1=20$  sec,  $T_2=150$  sec, and  $T_3=500$  sec. In the same figure, the sensitivity coefficients  $v_1(t)$ ,  $v_2(t)$ , and  $v_3(t)$  are presented. It is clear that the dynamics of the primary phase of post-rotational response is dominated by the long-time constant  $T_1$  of the semicircular canals. The sensitivity of the response to variations of  $T_2$  becomes significant at the end of the primary phase and increases further during the secondary phase. The sensitivity coefficient  $v_3$  is always small. Therefore, the parameter  $T_3$  can only be crudely estimated.

## RÉSUMÉ

Les principaux paramètres du réflexe vestibulo-oculomoteur (le gain ou reflectivité, la constante de temps majeure des canaux semicirculaires, la constante de temps de la transduction mécano neurale et celle de l'intégrateur centrale) ont été calculés à partir des réponses nystagmiques post-rotatoires pour des sujets souffrant de maladie de Meniere unilatérale. La comparaison avec les mêmes résultats obtenus chez des sujets normaux montre une diminution des deux premiers paramètres soit dans les réponses horaires que dans les réponses anti horaires. Au contraire les autres paramètres ne montrent pas des différences significatives. Vraisemblablement à la suite d'une phénomène de compensation centrale, un niveau élevé de symétrie a été observé chez sujets avec un maladie de Meniere datante de plusieurs années.

## ZUSAMMENFASSUNG

Die wichtigsten Parameter des vestibulo-okulären Reflexbogens (d.h. der Verstärkungsfaktor, die lange Zeitkonstante der Bogengänge, die Zeitkonstante der peripheren Adaptation und die des zentralen „leaky integrator“) wurden aus dem postrotatorischen Nystagmus von Patienten berechnet, die an einem unilateralen M. Meniere litten. Der Vergleich mit normalen Versuchspersonen zeigte eine Abnahme der ersten beiden Parameter sowohl für Drehungen im als auch entgegen dem Uhrzeigersinn. Dagegen ergaben die übrigen Parameter keine signifikanten Änderungen. Vorwiegend bei Patienten mit längerer Anamnese wurde eine größere Symmetrie beobachtet, dies ist vermutlich durch eine zentrale Kompensation zu erklären.

## REFERENCES

- Altmann, F. & Kornfeld, M. 1965 Histological studies of Meniere's disease. *Ann Otol Rhinol Laryngol* 74, 915.
- Anzaldi, E. & Mira, E. 1975 An interactive program for the analysis of ENG tracings. *Acta Otolaryng* (Stockh) 80, 120.
- Anzaldi, E., Mira, E., Schmid, R. & Stefanelli, M. 1975 TAIS: an interactive program for analysis of vestibular nystagmus. *J Clin Computing* 6, 107.
- Baerischi, A., Johnson, N. & Hanna, R. 1975 Theoretical and experimental determination of vestibular dynamics in caloric stimulation. *Biol Cybern* 20, 175.
- Barnes, G. R. & Benson, A. J. 1973 A model for prediction of the nystagmic response to angular linear acceleration stimuli. *NATO AGARD CCP* 1, A23, 1.
- Bock, C. & Bromm, B. 1977 A mathematical model of the ocular reflex after hemilabyrinthectomy in the cat. *Exp Brain Res* 28, 235.
- Hedgecock, L. D. 1968 Audiotmetric findings in Meniere's disease. *Otolaryngol Clin North Am* 15 (Supplement on Meniere's Disease), pp. 489-497.
- Jeanerod, M. & Schmid, R. 1978 La plasticité du réflexe vestibulo-oculaire. In *Neurobiologie de l'apprentissage* (ed. J. Delacour). Masson, Paris.
- Malcolm, J. 1973 Adaptation of the vestibulo-ocular system to rotation. *Adv Oto-Rhino-Laryngol* 19, 20.
- Mira, E., Schmid, R. & Stefanelli, M. 1975 Application of a mathematical model of the vestibulo-ocular system to the analysis of ENG tracings. *Acta Otorhino-laryngol Belg* 29, 381.
- Mira, E., Schmid, R. & Stefanelli, M. 1975 Clinical analysis of vestibularly induced eye movements on a mathematical model of the vestibulo-ocular system. In *Basic Mechanisms of Ocular Motility and Clinical Implications* (eds G. Lennerstrand & C. H. Markham). Springer-Verlag, Berlin, Heidelberg, New York.
- Precht, W., Shimazu, H. & Markham, C. H. 1966 The analysis of ENG tracings. *Acta Otolaryng* (Stockh) 80, 120.

- mechanism of central compensation of vestibular function following hemilabyrinthectomy *J Neurophysiol* 29 996
- Chind R 1975 Clinical application of a mathematical model of the vestibulo-ocular reflex *Polish Italian symposium on biomedical engineering* Jablonna September 1974 Polish Academy of Sciences Publ pp 23-59
- Chind R & Lardini F 1976 On the predominance of anti-compensatory eye movements in vestibular nystagmus *Biol Cybernetics* 23 135
- Chind R, Mira E & Stefanelli M 1975 Un modèle du système vestibulo-oculomoteur *Acta Oto-rhinolaryngol Belg* 29 9
- Chind R, Stefanelli M & Mira E 1971 Mathematical modelling: A contribution to clinical vestibular analysis *Acta Otolaryngol* (Stockh) 72 292
- Waltle J 1968 Electronystagmographic results in Meniere's disease *Otolaryngol Clin North Am* (Symposium on Meniere's Disease) pp 509-518
- Steer R W 1967 The influence of angular and linear acceleration and thermal stimulation on the human semicircular canal ScD Thesis MIT, Cambridge Mass
- Sugie N & Melvill Jones G 1971 A model of eye movements induced by head rotation *IEEE Trans SMC* 1 251
- Tomovic R 1963 *Sensitivity Analysis of Dynamic System* McGraw Hill New York
- Wilmot T J 1974 Vestibular analysis in Meniere's disease *J Laryngol* 88 295

E Mira MD  
Otorhinolaryngological Clinic  
University of Pavia  
I 27100 Pavia  
Italy

# COMPUTER CALCULATION OF MOVEMENT OF BODY'S CENTER OF GRAVITY

K Taguchi, M Iijima and T Suzuki

*From the Department of Otolaryngology Faculty of Medicine  
Shinshu University Matsumoto Japan*

(Received January 4 1977)

**Abstract** The movement of the body's center of gravity was calculated in normal subjects and in vertiginous patients by using a strain gauge platform system and a digital computer. The total length of the loci traced by the center of gravity in 40 normal subjects during normal standing for 1 minute was  $52.0 \pm 18.5$  cm with eyes open and  $81.5 \pm 52.3$  cm with eyes closed. The ratio of the total length with the eyes closed compared with that with the eyes open during normal standing for 1 minute was  $1.56 \pm 0.56$ . The time course of the length of the locus was calculated every 10 seconds and characteristic curves were obtained. The dependability of this test appeared to be fairly accurate and the usefulness of this technique for the diagnosis of vertiginous or ataxic diseases is recommended.

caused by accidental deflection occurring during the recording of the body's displacement such as can arise when the body sway, while standing, is disturbed by a lurch caused by subject's lack of concentration. The calculation area may then be too great in comparison with the possible minimal displacement.

The present study was carried out with computer assistance, with the aim of obtaining exact data on the movement of the body's center of gravity.

The righting reflex, characterized by Romberg's test, has been an important tool in neurological diagnosis. However, there is no exact criterion for judging the results of the righting reflex. How wide a margin should be allowed between normal and ataxic subjects? Are there any characteristics of body sway particular to the standing human suffering from vertigo? These are matters of concern to those conducting research on the body's center of gravity, which is one of the indicators by which the degree of instability is expressed.

Recently, recording of the displacement of the body's center of gravity by means of the stabilograph has been introduced. This method of calculating the displacement consists of measuring on paper the area of the printed trace obtained from the center of gravity. The most common defect of this method is that errors are liable to show up in the results,

## MATERIAL AND METHOD

The Static Sensonograph constructed by Saner Co. (Kyoto, Japan) using the strain gauge technique was used with a subject standing on the platform in order to obtain a continuous record of the extent of movement of the body's center of gravity (CG is the abbreviation of body's center of gravity) in the horizontal plane. The output from the sensonograph in terms of voltage was connected to a plotting unit which recorded the data at the same time supplied to a magnetic tape recorder. The data were then transferred into a digital computer (NEAC-2200) at Tokyo.

further analysis (Fig. 1).

The program for operation of the computer was written by Assembly Language and the object program for calculation of the locus was applied. The sampling time was 0.125 sec and the analysis time was one or two minutes. The calculated results were printed out in digital form every 10 sec.

The study concerned 40 healthy test subjects (20 men and 20 females) aged 16 to 30. Five subjects with vertigo underwent the same test. Each subject was placed on the platform with the feet together and was asked to maintain a steady posture. The following data were recorded: (1) time of standing, (2) total length of the locus, (3) ratio of the total length with eyes closed to that with eyes open, (4) time course of the length of the locus, (5) characteristic curves of the locus.

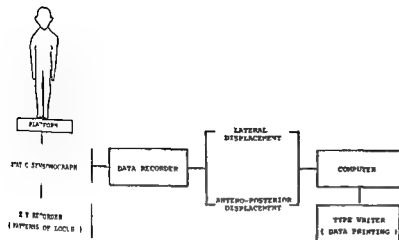


Fig. 1 Block diagram of equipment

## RESULTS

### Total length of locus traced by CG during standing

Table I shows the means and the upper rejection limits of the normal range in the total length of the locus traced by the CG during the first minute of standing. The difference in locus length obtained when standing with eyes open vis a vis with eyes closed was statistically significant in all conditions tested ( $p < 0.05$ ). In the different head positions with eyes open, the means with head inclined downward and upward were greater than with the other head positions ( $p < 0.05$ ). However

in the condition with eyes closed no differences among the values of the five head positions were revealed ( $p > 0.05$ ). In the data obtained by the Mann's positions, no statistical difference was found between the values with the right foot forward and those with the left foot forward ( $p > 0.05$ ). There were no differences between the values on the platform tilted in the four different planes ( $p > 0.05$ ).

### Ratio of total length of locus traced by CG with eyes closed compared with that with eyes open (EC/EO)

The gap in the length of the locus, traced by the CG with eyes open and that with eyes closed, would be considered to indicate the grade of optical compensation of the righting reflex in the body's stabilizing system. The

Table I Total length of locus traced by CG during 1 minute of standing ( $N=40$ )

	Head positions					Mann's positions		Platform 15° tilted			
	Straight	Head inclined				Right foot forward	Left foot forward	Right ward	Left ward	For ward	Back ward
		Towards the right	Towards the left	Down ward	Up-ward						
Means											
Eyes open	52.0	54.4	55.4	61.2	61.2	84.6	86.3	71.6	66.4	61.7	65.1
Eyes closed	81.5	79.5	78.4	78.8	88.0	161.4	159.9	104.7	106.9	98.5	100.9
Upper rejection limits ( $p=0.05$ )											
Eyes open	70.5	73.9	71.7	87.0	85.0	121.0	122.4	100.3	94.8	88.8	93.8
Eyes closed	133.8	133.4	121.0	123.6	139.1	225.4	226.0	166.4	167.5	155.5	157.5



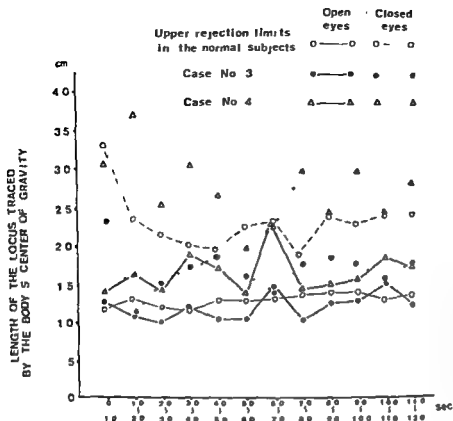


Fig. 2 Time course of the length of locus traced by the CG in the straight head position during the first one minute of standing. The time course of Case No. 3 with orthostatic hypotension and that of Case No. 4 with acoustic neuroma are shown with the upper rejection limits in the normal subjects ( $N=37$ ,  $p=0.01$ ).

smallest ratio (EC/EO) was obtained when the head was inclined downward while the largest ratio was obtained when the head was inclined forward (Table II).

#### Time course of length of locus traced by CG

The length of the CG locus in the straight head position for 2 min. was calculated every 10 sec (Fig. 2). The time course of the length of the locus with eyes open was very stable. However, a characteristic curve was obtained in the trials

with eyes closed, showing an initial large deflection followed by several small deflections.

#### Test-retest reliability

The length of the locus traced by the CG in the straight head position during the first one minute of standing was measured twice with an interval of one week on 32 subjects. The results calculated were  $52.0 \pm 18.5$  cm and  $50.3 \pm 18.0$  cm. There was no statistically significant difference between them ( $p > 0.05$ ).

Table II Ratio of the total length of locus traced by CG with eyes closed compared with eyes open: means  $\pm$  rejection limits ( $N=40$ )

Head positions	Head inclined				Mann's positions		Platform 15° tilted			
	Towards the right	Towards the left	Downward	Upward	Right foot forward	Left foot forward	Rightward	Leftward	Forward	Backward
Straight	$1.56 \pm 0.56$	$1.47 \pm 0.52$	$1.44 \pm 0.50$	$1.31 \pm 0.50$	$1.50 \pm 0.63$	$1.94 \pm 0.87$	$1.88 \pm 0.68$	$1.47 \pm 0.66$	$1.67 \pm 0.69$	$1.57 \pm 0.68$

Table III Total length of locus traced by CG (L) and ratios of the total length with eyes closed compared with that with eyes open (EC/EO) in the vertiginous patients during 1 minute of standing

A and N denote abnormal and normal values respectively as compared with the upper rejection limits in the normal subjects

	Head positions					Mann's positions		Platform 15° tilted				
	Straight	Head inclined				Right foot forward	Left foot forward	Right ward	Left ward	For ward	Up-ward	
		Towards the right	Towards the left	Down ward	Up-ward							
Case no 1												
L eyes open	A	A	A	A	A	A	A	A	A	A	A	
L eyes closed	A	A	A	A	A	A	A	A	A	A	A	
EC/EO	A	A	A	N	A	N	N	N	N	N	A	
Case no 2												
L eyes open	N	N	N	A	A	A	A	N	N	N	A	
L eyes closed	A	N	N	A	A	A	A	N	A	A	A	
EC/EO	N	N	N	N	N	A	A	N	N	A	A	
Case no 3												
L eyes open	N	N	A	A	A	N	N	A	A	A	A	
L eyes closed	N	N	A	A	A	N	A	N	N	A	N	
EC/EO	N	N	N	N	N	N	N	N	N	N	N	
Case no 4												
L eyes open	A	A	A	A	A	*	*	*	*	*	*	
L eyes closed	A	A	A	A	A	*	*	*	*	*	*	
EC/EO	N	A	N	A	A							
Case no 5												
L eyes open	A	A	A	A	A	*	*	*	*	*	*	
L eyes closed	A	A	A	A	A	*	*	*	*	*	*	
EC/EO	A	N	A	A	N							

\* Not measurable

**The practice of calculating the CG movement**  
To illustrate the clinical value of this method the results of 5 patients are described here (Table III)

**Case No 1** (62 year old female) She has been suffering from vertigo after sudden deafness in the left ear. Romberg's test and Mann's test were positive only with eyes closed. However, the calculated length of the locus of the CG was above the normal limit under the conditions with eyes open and with them closed.

**Case No 2** (23 year old female) A brain stem lesion was considered to be the cause of her instability. Abnormal results were obtained especially in the Mann's positions.

**Case No 3** (22 year old female) She had the typical symptoms of orthostatic hypotension. Romberg's test and Mann's test were negative. However, pathologic results were obtained with the computer.

**Case No 4** (56-year-old female) Acoustic neuroma in the left cerebello-pontine angle was suspected on the basis of otoneurologic tests and X ray examination. Recently the diagnosis was confirmed by surgical operation. She was too unstable to undergo the tests in Mann's positions and on the tilting platform.

**Case No 5** (36-year old male) He was in the active stage of Ménière's disease. Instability made it impossible for him to stand. Mann's test or the tilting platform test.

Table III shows the ratio of EC/EO of the above mentioned 5 patients. All cases except No. 3 showed abnormal values. Cases 1 and 5, with left sided peripheral vestibular lesions, had large values when the head was inclined towards the left.

The time course of the CG deflection was almost normal in the functional disease, such as in Case 3, while Case 4, with an organic lesion, presented a greatly fluctuating and irregular curve (Fig. 2).

## DISCUSSION

So far, many investigators have been engaged in quantitative measurement of the CG (Hellebrandt, 1938, Hellebrandt & Braun, 1939, Travis, 1944, 1945, Wapner & Witkin, 1950, Baron, 1964, Begbie, 1967, Baron et al., 1968, Njokiktjen & Folkerts, 1971, Kapteyn & de Wit, 1972, Benson & Jobson, 1973, Taguchi & Yoda, 1975).

In 1938 Hellebrandt estimated the maximal shifts in the center of gravity from kymographically recorded changes in a balance weight, which occurred when a subject stood on the pivot of series of platforms constructed on two scales concurrently (of du Bois-Jonod) placed at right angles to each other. Travis (1944) used a platform mounted on a universal joint. Wapner & Witkin (1950) used a stabilometer made of a wooden table upon which a subject stood while trying to keep it horizontal. Thomas & Whitney (1959) attempted to describe the postural sway in a more objective and mechanical manner, using a three dimensional platform equipped with a strain gauge system. Since then the strain gauge platform system has prevailed for recording the movement of the CG (Henriksson et al., 1966, Kapteyn & de Wit, 1972, Benson & Jobson, 1973, Taguchi, 1974). Begbie (1967) measured the postural sway by two potentiometers fixed to the pivots which supported the platform. Another device was used by Baron et al. (1968) and Njokiktjen & Folkerts (1971). They recorded the body motion

by using a four point square detector plate transforming variations of pressure into electric information by means of electromagnetic plungers.

The present research aims to calculate the exact value of the locus traced by the CG so that mutual comparison of the data between different stages in the disease of a patient and between normal people and patients suffering from vertiginous or ataxic diseases can be made.

In this study the amplification of our apparatus was adjusted so that the 100% product of body weight and body length of each test subject on the platform would give the same deviation of the tracing of the CG, in order to simplify the calculation of the results. The same device was adopted by Henriksson et al. (1966).

Our statistical analysis indicates that the length of the locus traced by the CG during a minute of standing showed a greater or lesser difference according to the head inclination, the foot positions and the platform tilt. Vertiginous or ataxic patients showed longer loci than did normal subjects.

EC/EO implies the grade of visual compensation for body balance (Wapner & Witkin, 1950, Kapteyn & de Wit, 1972). In our data the EC/EO of 4 vertiginous patients was outside the rejection limits for the normal subject. Using this indicator, it will be possible to differentiate vertiginous or ataxic diseases unaffected by eye closing, such as orthostatic hypotension and cerebellar ataxia.

The time course of the CG revealed the characteristic deflection in the length of the locus during standing with eyes closed. The initially large deflection and the subsequent several small deflections would be explicable by Nashner's model. When the ankle joint reflexes are active, postural stability will be achieved in preventing the body from diverging, resulting in an increase in depth sensation by exteroceptive and vestibular cues (Nashner, 1970).

The test-retest values showed that the de-

lity of this test was adequate for clinical use. These normal and clinical results suggest that the measurement of the CG movement seems to offer the possibility of being opened into a new diagnostic technique for cerebellar and ataxic patients.

## ZUSAMMENFASSUNG

Die Bewegung des Körperschwerpunktes wurde an normalen und pathologischen Versuchspersonen mittels eines optischen Sensorengraphen und einer elektronischen Recheneinheit berechnet. Die totale Länge des vom Körperschwerpunkt geschriebenen geometrischen Ortes an normalen Personen bei Stehen für eine Minute war  $52.0 \pm 18.5$  cm mit offenen und  $81.5 \pm 52.3$  cm mit geschlossenen Augen. Die Rate der totalen Länge mit geschlossenen Augen war bei denjenigen mit offenen Augen an normalen Personen bei Stehen für eine Minute war  $1.56 \pm 0.56$ . Der Verlauf der Bewegung des Körperschwerpunktes wurde jede Sekunde berechnet und die charakteristische Kurve wurde erzielt. Die Verlässlichkeit und die Gültigkeit von Messungen der Körperschwerpunktbewegung in klinischen Situationen wurde veranschaulicht.

## REFERENCES

- Baron J B 1964 Presentation d'un appareil pour mettre en évidence les déplacements du centre de gravité du corps dans le polygone de sustentation. Applications pratiques *Arch Mal Prof* 16: 8.
- Baron J B, Molinier J & Vniliac A 1968 Stato-kinesthetic recording of the body balance in sport medicine. *Medicine and Sport*, Vol 2 Biochemanics p 213. Karger Basel/New York.
- Berg G H 1967 Some problems of postural sway. *Symposium on myotatic kinesthetic and vestibular mechanisms* p 80. CIBA Found J & A Churchill London.

- Benson A J & Jobson P H 1973 Body sway induced by a low frequency alternating current. *Equilibrium Res* 3: 55.
- Hellebrandt F A 1938 Standing as a geotropic reflex. *Am J Physiol* 121: 471.
- Hellebrandt F A & Braun G L 1939 The influence of sex and age on the postural sway of man. *Am J Phys Anthropol* 24: 347.
- Henniksson N G, Johansson G, Olsson L G & Ostlund H 1966 Electric analysis of the Romberg test. *Acta Otolaryngol (Stockh)* Suppl. 224: 272.
- Kapteyn T M & de Wit H 1972 Posturography as an auxiliary in vestibular investigation. *Acta Otolaryngol (Stockh)* 73: 104.
- Nashner L M 1970 Sensory feedback in human posture control. Sc D Thesis MIT 70-3. Man-Vehicle Laboratory, Center for Space Research, MIT, Cambridge Mass.
- Njokiktjen Ch & Folkerts J F 1971 Displacement of the body's center of gravity at galvanic stimulation of the labyrinth. *Confin Neurol* 33: 46.
- Taguchi K 1974 The normal ranges of displacement in the center of gravity of the body. *Otolaryngol (Tokyo)* 46: 415.
- Taguchi K & Yoda M 1975 Effects of optokinetic stimulation on the center of gravity. *Proc of the Barany Society* 347.
- Thomas D O & Whitney R J 1959 Postural movements during normal standing in man. *J Anat* 93: 524.
- Travis R C 1944 A new stabilometer for measuring dynamic equilibrium in the standing position. *J Exp Psychol* 34: 418.
- 1945 An experimental analysis of dynamic and static equilibrium. *J Exp Psychol* 35: 216.
- Wapner S & Witkin H A 1950 The role of visual factors in the maintenance of body balance. *Am J Psychol* 63: 385.
- A. Taguchi MD  
Dept of Otolaryngology  
Faculty of Medicine  
Shinshu University  
1-1-3 Asahi  
Matsumoto Japan

## SYMPATHETIC NERVES AND NASAL SECRETION IN THE CAT

H Wilson and M S Yates

*From the Department of Pharmacology and Therapeutics  
University of Liverpool Liverpool England*

(Received July 14 1977)

**Abstract** The influence of sympathetic nerves on nasal secretion was examined in anaesthetized cats. The results from experiments in which the Vidian nerve was stimulated supramaximally for 3 min show that vasoconstriction occurs in the nasal cavity during the production of nasal secretion. The amount of nasal secretion produced in the normal cavity on Vidian nerve stimulation was greater than that produced in the sympathectomized nasal cavity but only significantly so at 2 and 5 Hz. It is concluded that at these lower frequencies sympathetic activity induced by Vidian nerve stimulation increases nasal secretion.

of vascular response. The amount of secretion evoked at both these parameters was also examined. In further experiments the secretion produced from the normal cavity on Vidian nerve stimulation was compared with that evoked from the sympathectomized nasal cavity.

Some of these findings have already been demonstrated to the Physiological Society (Wilson & Yates, 1976).

The Vidian nerve conveys preganglionic parasympathetic secretory, vasodilator and postganglionic sympathetic vasoconstrictor nerves to the nasal cavity of the cat (Malcomson 1973, Eccles & Wilson, 1973, Malm, 1973, Wilson & Yates, 1974). Supramaximal stimulation of the facial nerve in the brain stem evokes secretion in the nasal cavity (Eccles & Wilson, 1973). Similar amounts of nasal secretion were also produced by supramaximal stimulation of the Vidian nerve (Eccles & Wilson, 1973) although these stimulation parameters evoke vasoconstriction in the nose (Eccles & Wilson, 1974). In these studies secretion was measured after 3 min stimulation and vascular responses recorded only during 15 sec stimulation.

The present investigations were carried out on anaesthetized cats in order to examine the role of sympathetic nerves in nasal secretion. In preliminary experiments the Vidian nerve was stimulated at vasoconstrictor and vasodilator parameters (Eccles & Wilson 1974) to determine the extent and duration of each type

### METHOD

Cats of either sex, free from nasal infection and 2-4 kg body weight were anaesthetized with pentobarbitone sodium 40 mg/kg i.p. and the trachea cannulated.

Vascular changes in the nose were recorded as pressure changes in the sealed nasal cavity by means of a short plastic cannula which was inserted into the nostril and connected to a pressure transducer (Bell & Howell type 4327 223) and a Devices M2 pen recorder (Wilson & Yates, 1975). This method records only the overall vascular changes in the nose and mainly reflects the changes in the capacitance vessels (Anggård & Edwåll 1974, Malm 1974). Nasal secretion was collected from the nasal cavity by placing in the nostril a cannula connected to a pre weighed bottle to which continuous suction was applied (Eccles & Wilson, 1973). The recording of vascular responses and collection of nasal secretion were undertaken in separate experiments.

The Vidian nerve was exposed in the o

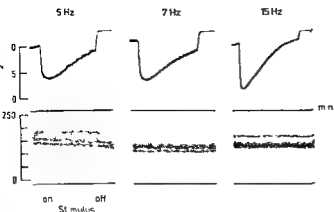


Fig. 1 Vasconstrictor responses evoked on supramaximal 3 min Vidian nerve stimulation. Lower record shows arterial blood pressure.

the method of Eccles & Wilson (1973) the cut peripheral end was placed on bipolar innum electrodes and covered with a small cotton wool swab soaked in liquid paraffin. The sphenopalatine and infraorbital nerves and cervical sympathetic chains were exposed. The Vidian nerve was also exposed in some cats in which the superior cervical ganglion had been removed 10–14 days previously and thus the Vidian nerve was purely asympathetic.

square wave impulses were delivered from Grass S4 stimulator through an isolation unit (U4). In secretion experiments the Vidian nerve was stimulated supramaximally (7.0 V) using a pulse width of 1.0 msec and at 2.0 V 1.0–2.0 msec. The nerve was stimulated for 3 min and allowed to rest for 7 min, the secretion being collected over a 10-min period. The rate of flow of secretion was expressed as mg/min.

In experiments in which vascular responses were recorded the Vidian nerve was stimulated at the same parameters for 3 min every 10 min.

In all experiments the arterial blood pressure was recorded from a femoral artery by means of a pressure transducer (Bell & Howell type 4327-221) and a pen recorder (Devices 2).

Non paired comparisons and Student's *t*-test were used in the statistical analyses.

## RESULTS

### *Duration of vasoconstriction and vasodilation produced in nasal cavity by Vidian nerve stimulation*

(a) *Vasoconstriction* Previous studies on Vidian nerve induced nasal secretion in the cat have shown that secretion appears between 2 and 5 Hz, increases with increasing stimulation frequencies, to reach a maximum value between 10 and 15 Hz (Eccles & Wilson, 1973).

The cut peripheral end of the Vidian nerve was therefore stimulated supramaximally in 4 cats for 3 min at 5, 7 and 15 Hz. The cats were given atropine sulphate (50 µg/kg i.v.) to prevent a pressure change in the nasal cavity caused by accumulation of nasal secretion which could interfere with the recording of vasoconstriction.

The results obtained from one cat (which are typical of those in the remaining three) are shown in Fig. 1. At all frequencies, vasoconstriction appeared immediately on stimulation to reach a maximum value at approximately 10 sec but the extent to which it occurred and the duration for which it was sustained depended on the stimulation frequency. Thus at 5 and 7 Hz it fell to between 60 and 70 mmHg initial value by 90 sec and to 60% at 3 min. At 15 Hz it declined rapidly reaching 70 mmHg

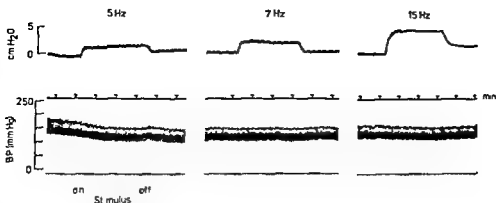


Fig 2 Vasodilator responses evoked on 3 min Vidian nerve stimulation at 2.0 V 0.2 msec. Lower record shows arterial blood pressure

response by 90 sec and it was undetectable after 3 min.

(b) *Vasodilation* Vasodilation was evoked at 5, 7 and 15 Hz in the nasal cavity of 4 cats by stimulating the Vidian nerve with parameters of 2.0 V and 0.2 msec. Parameters greater than this could not be used as they produce vasoconstriction.

Vasodilation appeared more slowly than vasoconstriction and increased with increasing stimulation frequency. In contrast to the vasoconstrictor responses, vasodilation remained constant throughout the 3 min stimulation period, returning slowly to the pre-stimulation level only after stimulation had ceased. The findings in one of these experiments are shown in Fig 2.

### Secretion studies

(a) *Secretion induced by parameters which induced vasoconstriction and vasodilation*

Nasal secretion was collected from each of 6 cats by stimulating the Vidian nerve at each of the vasoconstriction and vasodilator parameters at frequencies ranging from 2 to 25 Hz.

Using vasoconstrictor parameters watery nasal secretion appeared 30 sec after stimulation began. The minimal effective frequency was 2–5 Hz, the flow increasing with increased stimulation frequency to reach a maximum value of 10–20 Hz. The maximum rate

of flow of secretion in 6 cats ranged from 41 to 114 mg/3 min (mean  $83.4 \pm 11.4$  S.E.).

In these 6 cats, nasal secretion could only be evoked in 3 when using vasodilator parameters. When secretion did occur, it appeared after 30 sec between 2 and 5 Hz to reach a maximum value between 15 and 20 Hz. At each frequency the flow rates were only approximately 80% of those obtained with vasoconstrictor parameters.

(b) *Secretion in sympathectomized nasal cavity*

To investigate further the role of sympathetic nerves on the secretory activity of the nasal mucosa, nasal secretion evoked in 6 normal cats was compared with that obtained from the sympathectomized cavity of 6 cats by supramaximal Vidian nerve stimulation. The stimulation parameters only produced vasodilation in the denervated cavity.

A watery nasal secretion appeared after an interval of 30 sec at a frequency of 2 Hz from both the normal and the sympathectomized nasal cavity. In both instances the secretion increased with increasing stimulation frequency, to reach a maximum value at 15 Hz. These findings are shown in Fig 3. In order to eliminate the variation in body weight, secretion was expressed as mg/3 min/kg.

The mean values for secretion obtained

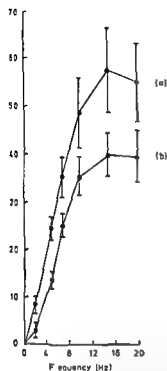


Fig. 3. Secretion frequency/response curves obtained in the normal cavity (a) the sympathetically denervated cavity on supramaximal 3 min Vidian nerve stimulation. Each value is the mean of those obtained in 6 cats (S.E. bars included).

from the normal cavity were greater than those from the sympathetomized cavity at all frequencies—but only significantly greater at 2 and 5 Hz ( $P < 0.05$ ).

## DISCUSSION

Previous studies have shown (Eccles & Wilson 1973, 1974) that stimulation of either the facial nerve in the brain stem of the cat, or the Vidian nerve, evokes similar amounts of nasal secretion. Whereas brain stem stimulation causes vasodilation in the nasal cavity, Vidian nerve stimulation induces vasoconstriction. These findings suggested that the accompanying vasoconstriction has little effect on the secretory activity of the nasal mucosa. The main criticism is however that secretion was

measured for 3 min, whereas the vascular responses were produced only by 15 sec stimulation.

When, however, the Vidian nerve was stimulated for 3 min at frequencies when secretion appears (5 Hz), when it is increasing (7 Hz) and reaches a maximum (15 Hz), vasoconstriction reaches its peak value within seconds. Vasoconstriction then declines in a frequency-dependent manner during the 3 min stimulation period. At all frequencies it is still present after 90 sec and only at 15 Hz was it undetectable after 3 min. Since nasal secretion appears in the cannula after 30 sec, vasoconstriction must be present during the secretory activity of the nasal glands.

In contrast, vasodilation evoked by weaker stimuli was sustained throughout the 3 min stimulation period. Maximum vasodilation could not be elicited because in crease of stimulus intensity produces vasoconstriction. The threshold of vasodilator fibres was apparently lower than that of secretory fibres because secretion occurred in only 50% of the cats using vasodilator parameters.

Supramaximal stimulation of the Vidian nerve to the sympathetomized cavity evoked only vasodilation. Nasal secretion appeared after the same latent period as in the normal cavity, indicating that the immediate vasoconstriction occurring in the normal cavity had no effect on the initial production of nasal secretion. The secretion/frequency response curves obtained from the normal and sympathetomized cavities were also similar. Although the mean values of secretion from the normal cavity were greater than those from the sympathetomized cavity at all frequencies, they were only significantly greater at 2 and 5 Hz, suggesting that at these frequencies sympathetic activity may aid the production of secretion.

These findings are in contrast to the histological studies of Dahlstrom & Fuxe (1966) who could not detect adrenergic fibres in the glands of the nasal mucosa of the guinea pig and rabbit although Å



Densert (1974) reported sparse adrenergic innervation of the nasal mucosal glands of the cat.

## ACKNOWLEDGEMENT

This work was supported by a United Kingdom Medical Research Council project grant to Dr H. Wilson

## ZUSAMMENFASSUNG

Der Einfluß von sympathischen Nerven auf die Nasensekretion wurde an narkotisierten Katzen untersucht. Supramaximale Reizung des Nervus vidianus für drei Minuten ergab ein Nasensekret und eine gleichzeitige Vaskonstriktion in den Blutgefäßen der Nasenhöhle. Die durch Vidianusreizung ausgelöste sezernierte Menge von Nasensekret in der normalen Nasenhöhle war größer als die, die bei diesen Reizfrequenzen in der sympathektomierten Nasenhöhle gemessen wurde, aber nur bei 2 und 5 Hz war der Unterschied signifikant. Daraus kann man schließen, daß bei diesen niedrigen Reizfrequenzen, die durch Reizung des Nervus vidianus hervorgerufene Nasensekretion von der gleichzeitigen Stimulation der in diesem Nerven verlaufenden sympathischen Fasern gesteigert wird.

## REFERENCES

Ånggård, A. 1974 The effects of parasympathetic nerve stimulation on the microcirculation and secretion in the nasal mucosa of the cat *Acta Otolaryngol* (Stockh) 78: 98

- Ånggård, A. & Densert, O. 1974 Adrenergic innervation of nasal mucosa in cat *Acta Otolaryngol* (Stockh) 232
- Ånggård, A. & Edwall, L. 1974 The effects of sympathetic nerve stimulation on the tracer disappearance rate and local blood content in the nasal mucosa: the cat *Acta Otolaryngol* (Stockh) 77: 131
- Dahlström, A. & Fuxe, K. 1965 The adrenergic innervation of the nasal mucosa of certain mammals *J Otolaryngol* (Stockh) 59: 65
- Eccles, R. & Wilson, H. 1973 The parasympathetic secretory nerves of the nose of the cat *J Physiol* (Lond) 230: 213
- 1974 The autonomic innervation of the nasal blood vessels of the cat *J Physiol* (Lond) 238: 549
- Malcomson, K. G. 1959 The vasomotor activities of nasal mucous membrane *J Laryngol Otol* 73: 73
- Malm, L. 1973 Stimulation of sympathetic nerve fibres in the nose in cats *Acta Otolaryngol* (Stockh) 73: 31
- 1974 Responses of resistance and capacitance vessels in feline nasal mucosa to vasoactive agents *Acta Otolaryngol* (Stockh) 78: 90
- Wilson, H. & Yates, M. S. 1975 Crossed sympathetic innervation of the cat nasal vasculature *J Physiol* (Lond) 247: 4P
- 1976 The role of sympathetic nerves in nasal secretion in the cat *J Physiol* (Lond) 258: 51P

H. Wilson, M.D. Ph.D.  
Department of Pharmacology and Therapeutics  
New Medical Building  
P.O. Box 147  
Ashton Street  
Liverpool L69 3BX  
England

## THE DOMESTIC PIG AS AN EXPERIMENTAL ANIMAL FOR STUDIES ON THE NASAL CYCLE

R Eccles

*From the Department of Physiology University College Cardiff Wales Great Britain*

(Received October 6 1977)

*Abstract:* The resistance to air flow of each nasal passage in the anaesthetized pig was determined by pumping air through the nose and measuring pressure changes in nasal cannulae. A nasal cycle was observed in the majority of the pigs. Three types of change in nasal resistance were observed and their possible causes are discussed. Section of the right cervical sympathetic nerve abolished the cyclic changes in nasal resistance of both nasal passages. The method described may prove useful in studying the physiology of the nasal cycle.

The phenomenon of the nasal cycle is well established in man (Heetderks, 1927, Stoksted 1953) and more recently cyclic changes in nasal resistance have been demonstrated in laboratory animals (Bojsen Møller & Fahrenkrug 1971, Eccles & Maynard, 1975). The nasal resistance is determined by the state of engorgement of the erectile tissue in the nasal mucosa and this is controlled by the autonomic nervous system. There is some evidence that the nasal cycle is regulated through the sympathetic innervation of the nose (Beickert, 1950, Stoksted & Thomsen 1953) and this is based on studies involving local anaesthesia of the stellate ganglion in man. Since the period of the nasal cycle is between 0.5-4 hours (Heetderks, 1927) and the duration of the local anaesthesia is only about 2 hours it is difficult to interpret whether or not the procedure has abolished the nasal cycle. In the present study the pig has been used as an experimental model to study the role of the sympathetic innervation in the regulation of nasal resistance. The anatomy of the nasal cavity and the structure of the nasal mucosa of the pig are very similar to man (Melon 1968) and the methods

used in the present study may prove useful in future studies to determine the function of the nasal cycle in man.

### METHODS

Thirteen 'Large White' pigs were used for the experiments (6 female, 6 male, 1 castrate male, body weights 16-50 kg). The pigs were anaesthetized with 'Halothane' through a face mask, then the trachea was intubated and the pigs maintained on closed circuit Halothane anaesthesia. Each pig was anaesthetized for a 4-5-hour period at intervals of 7-14 days. In every experiment nasal resistance was recorded during the period 10.00-16.00 hours. The pigs were rested on a thick foam rubber mat to prevent pressure sores and then covered with an electric blanket to maintain the body temperature at 39°C. The range of laboratory temperature throughout the period of the experiments was 18-22°C with a range of relative humidity of 25-50% saturated.

The resistance to air flow of each nasal passage was determined by means of a twin cylinder pump which moved air through the nasal passages as shown in Fig. 1. The posterior nares were cannulated with a 10 mm internal diameter rubber tube with a shaped copper end piece to ensure a free flow of air from the nose to the mouth. Specially designed plastic cannulae were fixed to the nostrils by means of a slight vacuum over the skin surrounding the nostril and an airtight seal was easily made on the smooth surface of the pig's nose. The pump was driven at 29 r.p.m. by means of

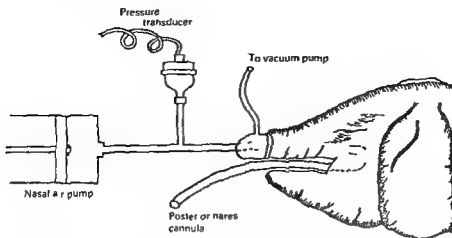


Fig 1 Diagram of determining nasal resistance, showing the position of the pump and cannulae

electric motor and a volume of 84 ml was moved by each piston stroke. Pressure transducers of the type used to monitor arterial blood pressure (Consolidated Electronics type 4 327 L221) were connected to side arms from each nasal cannula (see Fig 1), and the pressure signals were displayed on a 'Devices' four-channel pen recorder. The change in pressure in the nasal cannula with each stroke of the pump was directly related to the resistance to air flow of the nasal passage. The pressure transducers were calibrated with a water manometer at the start and end of each experiment and the two cylinders of the pump and the nasal cannulae were regularly checked with standard resistances to ensure that each half of the pump and recording system was identical.

The respiratory rate, heart rate and body temperature were recorded at 30 min intervals throughout the experiment. The respiration was also continuously monitored by means of a mercury in rubber strain gauge strapped around the chest of the pig. The signal from the strain gauge was displayed on the pen recorder trace.

#### *Section of the cervical sympathetic nerve*

The right cervical sympathetic nerve was sectioned under Halothane anaesthesia. The nerve was identified by monitoring nasal resistance (as above) and noting the effects of clamping, section, and electrical stimulation of the

cut peripheral end of the nerve. These procedures should cause nasal vasoconstriction and a decrease in the resistance of the ipsilateral nasal passage if the sympathetic fibres to the nose are present in the nerve.

### RESULTS

The method described for determining nasal resistance has proved successful and has been developed over a period of 3 years of experimentation with different techniques. The major problems encountered in the development of the method have been the cannulation of the nostrils and the maintenance of a free airway from the posterior nares to the mouth. These problems have been overcome by the development of special suction cannulae to attach to the nostrils and a specially designed posterior nares cannula.

At the start of the recording period one nasal passage usually had a high resistance to air flow when compared with the other and the resistance of each nasal passage gradually increased during the first hour of recording. Spontaneous changes in nasal resistance were recorded in all of the pigs and a typical example of the changes observed in one pig is shown in Fig 2. These were usually slight increases in nasal resistance of 1-5 min duration (see right nasal passage, Fig 2) accompanied by changes in the depth and rate of respiration and slight movements of the limbs which could

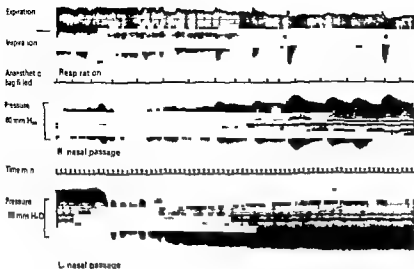


Fig 2 Pen recorder trace of pressure changes in nasal cannulae and changes in respiration. Spontaneous changes in nasal resistance are apparent in both nasal passages (see text) (Pig no 36)

not be related to any change in the depth of anaesthesia or to changes in the concentration of carbon dioxide in the inspired and expired gas in the anaesthetic circuit. The spontaneous changes in nasal resistance were often repeated at regular intervals with regular changes in respiration.

In 10 of the 13 pigs reciprocal changes in nasal resistance occurred with the resistance of one nasal passage increasing as the resistance of the other passage decreased (see Fig 3). The reciprocal changes in resistance started at the same time in each nasal passage and oc-

curred over a period of approximately 30 min. These changes in resistance occurred only once or twice during the 4–5 hour recording period and did not usually occur in the first hour of recording. This reciprocal activity of the nasal mucosa was observed in 25 out of 31 experiments on 10 pigs.

In 4 of the pigs nasal resistance was monitored on three occasions before section of the right cervical sympathetic nerve and on two occasions after nerve section. In 3 of the pigs, clamping of the nerve or electrical stimulation of the cut peripheral end caused a marked de-

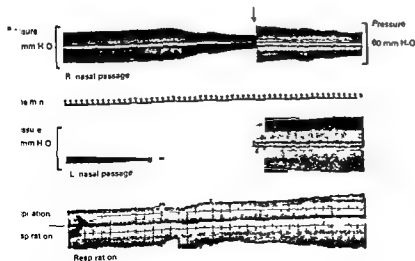


Fig 3 Pen recorder trace of pressure changes in nasal cannulae and changes in respiration. Reciprocal changes in nasal resistance are apparent (see text). Note change of gain for the upper trace point marked with .

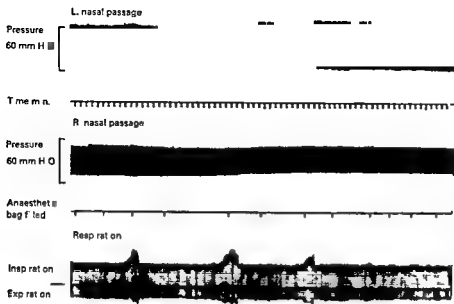


Fig. 4 Pen recorder traces showing pressure changes in nasal cannulae and changes in respiration taken from an experiment 111 days after section of the right cervical sympathetic nerve (Fig. 1, 36). Compare with traces from preoperative experiments in Figs. 2 and 3.

crease in the resistance of the ipsilateral nasal passage, and in two of the pigs a slight decrease in the resistance of the contralateral nasal passage was also observed. In the fourth pig electrical stimulation of the cervical sympathetic nerve caused only a slight decrease in the resistance of the ipsilateral nasal passage and had no effect on the contralateral passage and it is probable that the majority of the sympathetic fibres to the nose escaped section in this pig. In the preoperative experiments,

spontaneous changes in nasal resistance were observed and reciprocal changes in nasal resistance were recorded in 9 out of 12 experiments on the 4 pigs. All four pigs develop post operative ipsilateral ptosis and hypermia of the ear. After nerve section no reciprocal changes in nasal resistance were observed and spontaneous changes in nasal resistance were rare and usually limited to the nasal passage contralateral to the nerve section. Fig. 5 is a typical record of the nasal resistance after

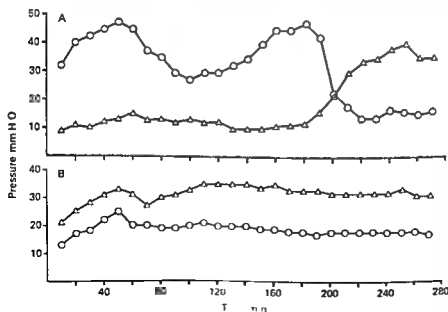


Fig. 5 Graphs to illustrate changes in nasal resistance: (A) before section of the right cervical sympathetic nerve; (B) 28 days after nerve section (Fig. no. 36). O, Right nasal passage;  $\Delta$ , left nasal passage.

nerve section and it should be compared with Figs 2 and 3 which are records from the same pig before nerve section. The gradual increase in the resistance of both nasal passages during the first hour of recording still occurred after nerve section yet after this change the resistance of each passage was relatively constant. Fig. 5 compares the changes in resistance observed before and after nerve section and demonstrates the low resistance of the right nasal passage after the operation which is typical of the results as no chronic increase in nasal resistance was observed on the right side.

### DISCUSSION

The results demonstrate that changes in nasal resistance may be recorded in the anaesthetized pig by pumping air through the nasal passages and recording pressure changes in nasal cannulae. The pump moves air to and fro in the nasal passages and this overcomes the problem of drying of the nasal mucosa which could occur with a continuous flow of air. The anterior nares cannula formed a very low and constant resistance in series with the two nasal passages and this ensured that changes in the pressure recordings from the nasal cannulae were directly related to the state of engorgement of the erectile tissue of the nasal mucosa.

Three types of activity were noted in the recordings of nasal resistance. Type (1) was a gradual increase in the resistance of each nasal passage over the first hour of recording. Type (2) was spontaneous increases or decreases in nasal resistance often with a regular periodicity of 5–10 min and usually associated with changes in respiration. Type (3) was a reciprocal activity with the resistance of one nasal passage increasing as the resistance of the other side decreased.

The gradual increase in nasal resistance (type 1) was observed in all of the pigs and this may be due to condensation of water within the nasal passages as during the experiment the air within the pump became saturated with

water and it was necessary to drain the cannulae of condensation every 2 hours. The increase in nasal resistance might also be explained by some action of the anaesthetic agent.

The spontaneous changes in nasal resistance (type 2) were accompanied by changes in respiration. Changes in nasal resistance in man have been shown to occur in an integrated way with changes in respiration in response to exercise and changes in arterial  $p\text{CO}_2$  (Dallimore & Eccles 1977) and it appears that the respiratory centre may be closely linked to other areas of the brain stem which regulate the activity of the autonomic innervation of the respiratory tract. Thus one would expect that changes in respiration would be accompanied by changes in nasal resistance. The changes in respiration in the pig may be due to depression of the respiratory centre by the anaesthetic agent and a periodic escape from this depression due to gradual build up of carbon dioxide. However any build up of carbon dioxide would be expected to cause a simultaneous decrease in resistance of each nasal passage (Tatum 1923; Dallimore & Eccles 1977) and this was not observed in the present experiments. Therefore the results are not fully explained by this hypothesis.

The reciprocal changes in nasal resistance (type 3) have been previously reported to occur in the pig (Eccles & Maynard 1975) and are similar to those observed in the human nasal cycle (Stoksted 1953). The changes in resistance are believed to be regulated by changes in the activity of the autonomic innervation of the erectile tissue and this activity is regulated from a nasal centre in the medulla or hypothalamus (Stoksted 1953; Eccles 1977). There is some evidence that the sympathetic nervous system has a major role in regulating the nasal cycle (Stoksted & Thomsen 1953) and this is based on local anaesthesia of the stellate ganglion in man. In the present study complete section of the cervical sympathetic nerve was possible and recordings made on the pigs before and after dec-

tion of the superior cervical ganglion. Section of the right cervical sympathetic nerve abolished the reciprocal changes in nasal resistance and almost completely eliminated the spontaneous changes in nasal resistance, in both nasal passages. The bilateral effect of nerve section may be explained by crossover of sympathetic fibres (Wilson & Yates, 1975) although only a fraction of the sympathetic fibres appear to cross over in the pig. Another possibility is that sensory fibres have a role in regulating nasal resistance and that ipsilateral nerve section causes a bilateral disturbance. Immediately after nerve section there was a gradual increase in the resistance of the ipsilateral nasal passage indicating a constant sympathetic tone. A similar result has been reported in man (Beickert, 1950; Stoksted & Thomsen, 1953). However, nerve section did not cause a chronic increase in nasal resistance in the pigs and the development of decentralization supersensitivity may explain the low resistance observed in the right nasal passage in post operative experiments (Malm, 1974).

### ACKNOWLEDGEMENTS

Thanks are due to Mr H. L. Nash and Mr H. J. Teagle for technical assistance and to the Mechanical Workshop of the Department of Physiology for the construction of the nasal pump and cannulae.

### ZUSAMMENFASSUNG

In den beiden Nasengängen narkotisierter Schweine wurde der Widerstand gegen einen Luftstrom gemessen. In dem Luft durch die Nase gepumpt und Druckänderungen in Nasenkanülen gemessen wurden. Bei der Mehrzahl der Schweine wurde ein Nasenzyklus beobachtet. Es wurden drei Typen von Nasenwiderstands-Veränderungen beobachtet, deren mögliche Ursachen besprochen wer-

den. Ein Durchschneiden des rechten III. sympathicus cervicalis führte zu einer Aufhebung der zyklischen Veränderungen des Nasenwiderstandes in den beiden Nasengängen. Die beschriebene Methode könnte sich für die Untersuchung der Physiologie des Nasenzyklus als wertvoll erweisen.

### REFERENCES

- Beickert P. 1951. Halbseitenrhythmus der vegetativen Innervation. *Arch. Ohren Nasen Kehlkopfheilkd.* 137, 404.
- Bojsen Møller F. & Fahrenkrug J. 1971. Nasal wall bodies and cyclic changes in the air passages of the rat and rabbit nose. *J. Anat.* 110, 25.
- Dallimore N. S. & Eccles R. 1977. Changes in human nasal resistance associated with exercise, hyperventilation and rebreathing. *Acta Otolaryngol. (Stockh.)* 84, 416.
- Eccles R. 1977. Cyclic changes in human nasal resistance to air flow. *J. Physiol.* 272, 75.
- Eccles R. & Maynard M. L. 1975. Studies on the nasal cycle in the immobilized pig. *J. Physiol.* 247, 1.
- Heidericks D. L. 1927. Observations on the reaction of normal nasal mucous membrane. *Amer. J. Med. Sci.* 174, 231.
- Malm L. 1974. Sensitivity of the nasal vessels of the cat to chemical agents after sympathetic and parasympathetic denervation. *Acta Otolaryngol. (Stockh.)* 77, 34.
- Mélon J. 1968. Contribution à l'étude de l'activité sécrétoire de la muqueuse nasale. *Acta Otolaryngol. Belg.* 22, 1.
- Stoksted P. 1953. Rhinometric measurements for the determination of the nasal cycle. *Acta Otolaryngol. (Stockh.)* Suppl. 109, 159.
- Stoksted P. & Thomsen K. A. 1953. Changes in the nasal cycle under stellate ganglion block. *Acta Otolaryngol. (Stockh.)* Suppl. 109, 176.
- Tatum A. L. 1923. The effect of deficient and excessive pulmonary ventilation on nasal volume. *Amer. Physiol.* 65, 229.
- Wilson H. & Yates M. S. 1975. Crossed sympathetic innervation of the rat nasal vasculature. *J. Physiol.* 247, 1.

R. Eccles Ph.D.  
Dept. of Physiology  
University College  
Cardiff  
Wales  
Great Britain

## THE NASAL MUCOSA DURING LONG-TERM TREATMENT WITH BECLOMETHASONE DIPROPIONATE AEROSOL

### *A Light and Scanning Electron Microscopic Study of Nasal Polyps*

N Mygind, H Sørensen and C B Pedersen

*From the University ENT clinics Rikshospitalet and Kommunehospitalet Copenhagen Denmark*

(Received June 5 1977)

**Abstract** Blind examination by light and scanning electron microscope of a total of 142 biopsies from nasal polyps was carried out before and after continuous intranasal treatment with beclomethasone dipropionate aerosol 400 µg/day for a period of 9-36 months. The degree of tissue oedema, the number of infiltrating eosinophil cells and the number of goblet cells in the epithelium decreased significantly. There was no change in the type of surface epithelium during treatment and no tendency towards squamous cell metaplasia. The study suggests that beclomethasone dipropionate treatment for a period of a few years will not cause atrophic rhinitis.

Since the introduction of beclomethasone dipropionate (Bdp) aerosol on the British and the European market 2-4 years ago it has become a well established and widely used drug for the management of hay fever, perennial rhinitis and nasal polyposis. A series of reports, demonstrating the efficacy of this treatment have appeared (reviewed by Brogden et al, 1975). As the active component is a highly potent corticosteroid with topical activity, it is obvious that the potential risk of local side effects needs consideration, and a study of the Bdp treated mucous membranes very relevant.

Dermatological experiences with skin atrophy following corticosteroid treatment suggest that the development of atrophic rhinitis following intranasal Bdp therapy is a possibility and this worries otorhinolaryngologists. A characteristic feature of atrophic rhinitis is squamous cell metaplasia of the surface

epithelium, demonstrable by both the light (Holopainen, 1967) and the scanning electron microscope (Mygind et al, 1974a).

This article presents the results of light and scanning electron microscopy of the nasal mucosa before and after 9-36 months' treatment with Bdp.

### MATERIAL & METHODS

Following a blind short term investigation of the efficacy of intranasal Bdp in adults with nasal polyposis (Mygind et al, 1975) 33 out of 35 patients continued for a 9-12 months controlled period (Pedersen et al, 1976) with 400 µg Bdp/day as the only treatment. Nasal biopsies were taken before and on the last day of the treatment period. 21 were treated for 9 months and 12 for 12 months. Final biopsies were taken after 36 months in 9 patients who had used Bdp continuously in a daily dosage of 200-400 µg.

Two 4-8 mm large mucosal biopsies were taken by forceps or snare from an anterior polyp. Local analgesia was not used. All biopsies were immediately fixed in a 4°C cold fixative which in the case of light microscopy, was a buffered 4% formalin solution. The specimens were embedded in paraffin and 4-6 µm thick sections cut. Haematoxylin and



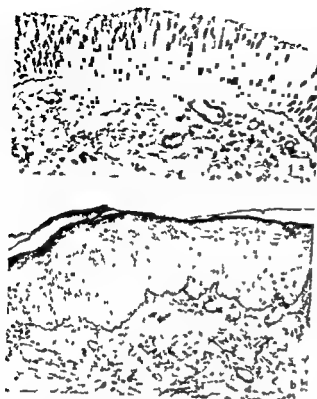


Fig 1 Light microscopic illustration of a pseudostratified epithelium (a) and a squamous epithelium (b) which correspond to score 100 and score 0 respectively in the blind examination of nasal biopsies before and after beclomethasone dipropionate treatment for one year

amine dyed sections were prepared from each specimen

After fixation for 1–7 days in 5% glutaraldehyde in 0.03 M sodium cacodylate (osmolality measured to 580 mOsm/kg) the biopsies for scanning electron microscopy were transferred to 0.15 M sodium cacodylate (osmolality measured to 295 mOsm/kg). They were then passed through a series of acetone solutions from 10–100% and transferred to liquid CO<sub>2</sub>. In an E 3000 Polaron critical point drying apparatus. After drying the specimens were mounted on stubs and a conductive layer of gold deposited on them in a vacuum evaporator. Finally the dehydrated gold coated specimen was studied in a Cambridge StereoScan S2 or a Jeol JSM 35 microscope. All biopsies except those taken after 36 months of

treatment, were coded and examined blind in both the light and the scanning electron microscope

A light microscope description of the appearance of the blood vessels and the glands was made based on haematoxylin–eosin and PAS stained sections and a description of the epithelial basement membrane was based mainly on the silver staining of the reticular fibrils. In elastin and in van Gieson sections only traces of elastic and collagen fibrils were found in the polyp tissue, so our study cannot give any information about possible Bdp-induced changes in these structures. The degree of tissue oedema was evaluated on a semi-quantitative scale (+ = 1, ++ = 2, +++ = 3). All eosinophils within one square millimeter were counted and so were the goblet cells in 1 mm of the surface epithelium. Based on the type of the surface epithelium each biopsy was given a score value from 0 (all epithelium of the squamous type) to 100 (all epithelium of the pseudostratified type) (Figs 1, 2)

In the scanning electron microscope a short description of the mucosal surface was given and two low power ( $\times 800$ ) and two high power ( $\times 3200$ ) micrographs of representative areas were taken. The description and micrographs of each biopsy were used to give a score value before the code was broken. The score system used is illustrated in Figs 3 and 4. Interpolation between the scores illustrated was necessary, when a biopsy was covered by

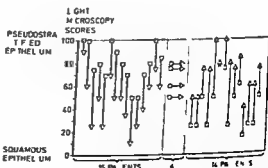


Fig 2 Results of blind light microscopic examination of nasal biopsies before ( $\square$ ) and after beclomethasone dipropionate treatment ( $\Delta$ )

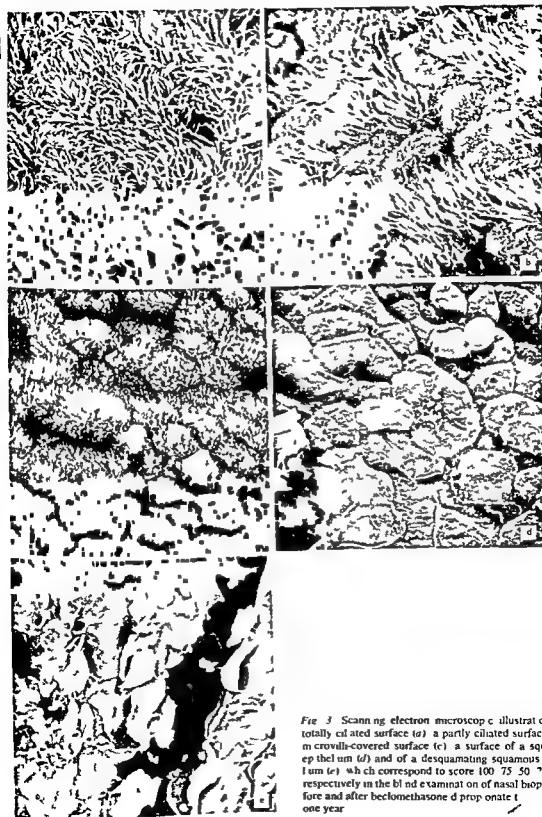


Fig. 3 Scanning electron microscopic illustration of a totally ciliated surface (a) a partly ciliated surface (b) a mucus-covered surface (c) a surface of a squamous epithelium (d) and of a desquamating squamous epithelium (e) which correspond to score 100 75 50 75 and respectively in the blind examination of nasal biopsy before and after beclomethasone dipropionate treatment one year.

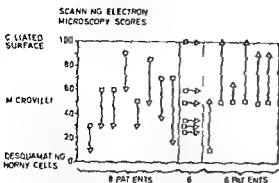


Fig 4 Results of blind scanning electron microscopic examination of nasal biopsies before ( $\square$ ) and after ( $\Delta$ ) beclomethasone dipropionate treatment

epithelia of varying character. Due to this the grading seems very detailed and exact but in fact it was merely semiquantitative.

For statistical calculations a paired Wilcoxon sign rank test was used. Only for the number of eosinophils and goblet cells the figures were transformed into logarithms. In no case did the result of a Wilcoxon test differ significantly from that of a paired *t*-test.

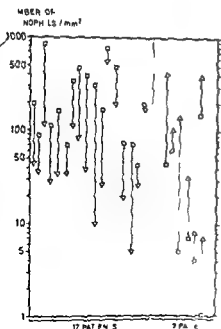


Fig 5 Number of eosinophils in nasal tissue before ( $\square$ ) and after ( $\Delta$ ) beclomethasone dipropionate treatment

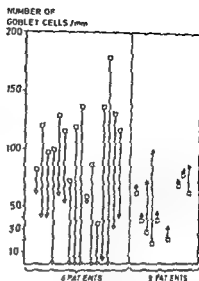


Fig 6 Number of goblet cells in nasal epithelium before ( $\square$ ) and after ( $\Delta$ ) beclomethasone dipropionate treatment

## RESULTS

Thirty-three pairs of biopsies were fit for light microscopy and 20 pairs for scanning electron microscopy. Some biopsies were discarded because, one or both of a pair did not contain enough epithelium, the biopsy was covered by mucus, or for other technical reasons.

Light microscopy showed the epithelial basement membrane to be of varying thickness without any consistent changes during the treatment period. The appearance of the sparse blood vessels did not change significantly during treatment and no perivascular haemorrhages were observed. Before and after treatment most glands were pathological containing only a few cystically dilated and degenerated branches. The huge amounts of tissue oedema decreased significantly during Bdp treatment ( $p < 0.05$ ) (Table 1) and so did the number of infiltrating eosinophils in lamina propria ( $p < 0.02$ ) (Fig 5), and also the number of goblet cells in the epithelium ( $p < 0.01$ ) (Fig 6).

Figs 2 and 4 show the changes in the type of surface epithelium from the pretreatment to the post-treatment biopsy within the single pairs. While the epithelium in 23 pairs of biopsies changed in the direction of a squamous epithelium, it changed in the direction of a



Fig 7 Scanning electron micrograph of a dry surface with many epithelial cells expelled. For further interpretation refer to text

pseudostratified epithelium in 20 pair of biopsies and no change occurred in 10 pairs. This blind, quantitative study did not disclose any consistent tendency towards the development of squamous cell metaplasia. A single post-treatment biopsy deserves further attention for as seen in Fig 7 the surface of this biopsy appeared "unclean" and dry with many cells expelled. Bdp may possibly account for this as this patient had dryness of the mucosa in the anterior part of the nasal cavity following treatment.

The biopsies taken after 36 months treatment did not differ significantly from the pre-treatment biopsies with regard to the type of epithelium but this examination was not blind.

## DISCUSSION

The significant reduction in tissue oedema of nasal polyps following Bdp treatment is consistent with the clinical observation that long term treatment has some shrinking effect on polyps (Pedersen et al 1976). As systemic corticosteroid therapy considerably diminishes the number of nasal eosinophils in hay fever (Mygind & Johnsen 1977) it is probable

that an equal effective, but topically administered, corticosteroid has a less pronounced effect on polyp eosinophils. This is in accordance with the observation that topical treatment produces only a slight, if any, reduction of eosinophilia in nasal smears (Sørensen et al 1976, Malm & Wihl 1976). It seems probable that Bdp is active only in the superficial layers of the mucosa with little effect on the cell diapedesis through the blood vessels.

The decreased number of goblet cells following Bdp treatment, may be a direct effect of the topically applied corticosteroid, but it seems more likely that the increased nasal patency during treatment is the direct cause. Proliferation of goblet cells is evoked by nasal blockage (Hilding & Hilding, 1970), and in the anterior part of the nose the number of goblet cells is found to be significantly higher in perennial rhinitis patients, especially in patients with pronounced nasal blockage than in normal subjects (Mygind et al 1974).

Neither light microscopy nor scanning electron microscopy of a total of 142 nasal biopsies showed a change of the surface epithelium from a pseudostratified to a squamous type. Thus microscopic studies suggest that continuous Bdp treatment, for up to 2½ years does not induce atrophic rhinitis. This is in accordance with the rhinoscopic findings in patients (Pedersen et al 1976).

It might seem hazardous to draw such conclusions on small mucosal biopsies for the character of the epithelium varies considerably from area to area within the nasal cavity (Mygind 1977). However single mucosal

Table I Tissue oedema before and after beclomethasone dipropionate treatment for one year

Degree of tissue oedema (+ = 1 ++ = 2 +++ = 3)	Mean of pretreat- ment values	Mean of posttreat- ment values
	2.4	1.6

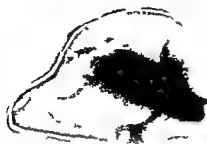


Fig. 8 Using a cast of a human nose it is illustrated that the distribution of a drug delivered from a metered dose freon propelled pressurized aerosol is uneven as it hits the mucosa like a bullet from a gun.

biopsies from patients with atrophic rhinitis have shown epithelial pathology (Holopainen, 1967, Mygind et al., 1974a). Therefore, we feel that the large number of biopsies studied would have disclosed any significant drug induced changes. Based on morphologic studies of polyp surfaces (Mygind, 1977) it is likely that the results obtained with polyp epithelium can be applied to the ordinary nasal epithelium.

The cause of spontaneously developing atrophic rhinitis is unknown, although it has been demonstrated that a lack of antiproteolytic enzymes might be of significance for the tissue damage (Reichert & Hochstrasser, 1972). It is common knowledge that extensive space-creating operations such as turbinectomy and ethmoidectomy may cause squamous cell metaplasia and intranasal crusting. It is also known that nasal polyps, due to pressure, can break the nasomaxillary suture and increase the distance between the septal and the lateral wall of the nose. Such a transformation of the normal slit like cavity to a broad tube will invariably increase the risk of squamous cell metaplasia and crusting. Consequently, any effective treatment of severe nasal polyposis may indirectly involve a risk of atrophic rhinitis.

Chronic nasal blockage favours pseudo-stratified epithelium (Mygind et al., 1974b) and prevents the development of atrophic rhinitis, but is intolerable to the patient. When improvement of nasal patency is needed it seems reasonable to suggest that conservative surgical treatment (polypectomy) followed by controlled Bdp therapy involves less risk of atrophic rhinitis than a radical surgical procedure such as ethmoidectomy.

Corticosteroids affect mainly connective tissue and the direct effect on epithelium is weak or absent (Azarnoff, 1975). One would expect should it occur, a Bdp induced atrophic rhinitis to be due to the steroid's effect on the lamina propria producing squamous cell metaplasia in the presence of an abnormally patent nasal airway. The initial step in such a process could not be studied by us as the polyps only contained traces of connective tissue fibrils in the lamina propria. However, recent transmission electron microscopic study of the connective tissue fibrils beneath the pharyngeal and the bronchial surface epithelium of asthma patients treated with Bdp for 12–18 months did not show any quantitative or qualitative abnormalities following treatment (Andersson et al., 1977).

Although neither rhinoscopic nor microscopic studies have suggested the development of atrophic rhinitis, some Bdp-treated patients may develop dryness of certain areas of the nasal mucosa and also a single crust (Petersen et al., 1976). In our experience these changes have not been serious and have been reversed, once the treatment has been stopped. These changes which may be followed by haemorrhagic spotting occur on the anterior part of the septum, on the front edge of the inferior and the middle turbinates and on the anterior surface of an anterior polyp. As illustrated in Fig. 8 these are the areas receiving the impact of the aerosol. The uneven distribution of a drug, delivered from a pressurized aerosol may also imply that other areas of the mucosa get subtherapeutic doses of it. Therefore, it may be worthwhile considering other

modes of administration and delivery systems for Bdp (Mygind, 1977)

Until now microscopic studies of the nasal pharyngeal and bronchial mucosa have not disclosed any serious local side effect following topical Bdp treatment (Andersson et al, 1977, Brain & Poynter, 1977, Jorde & Werdermann, 1977, Thiringer et al, 1975). Investigations of the nasal and the pharyngeal mucosa may be of special importance, for the steroid dosage per square centimeter is at least 100 times greater in these areas than in the bronchi. Therefore, any local side effects are likely to appear first in the nasal or pharyngeal mucosa. As inspection of the nasal mucosa is easy, it should be carried out regularly in all patients on continuous Bdp treatment both as a safety factor and to increase our knowledge of topical corticosteroids' action on the airway mucosa.

## ACKNOWLEDGEMENTS

The scanning microscopes were kindly placed at our disposal by the Institute for Historical Geology and Paleontology Copenhagen (H. J. Hansen/M.Sc.) and the Department of Pathology Hvidovre Hospital (P. Christoffersen/M.D.). The specimens for light microscopy were prepared with skill by Mrs J. Nørgaard Glaxo Copenhagen kindly supplied all aerosols used.

## ZUSAMMENFASSUNG

142 nasale Polypen wurden blind vor und nach einer 36 monatigen intranasalen Behandlung mit Beclomethasone dipropionate aerosol 400 µg/tgl. licht und elektronenmikroskopisch untersucht. Der Grad des Gewebsödems sowie Anzahl an eosinophilen Zellen und epithelialen Becherzellen waren deutlich herabgesetzt. Während des Behandlungsverlaufes konnte man keinerlei Veränderungen des Oberflächenepithels und keinerlei Tendenz zur Plattenepithelmetaplasie konstatieren. Aus der Untersuchung kann man schließen, daß eine durch wenige Jahre verabreichte Behandlung mit Beclomethasone dipropionate nicht zu atrophischer Rhinitis führt.

## REFERENCES

- Andersson E, Smidt C M, Sikjaer B, Ainge G & Poynter D 1977 Bronchial biopsies after beclomethasone dipropionate aerosol. *Brit J Chest Dis* (in press).
- Azarnoff D L 1975 *Symposium on steroid therapy*. Saunders Philadelphia.
- Brain D J & Poynter H 1977 Personal communication.
- Hilding D A & Hilding A C 1970 Electron microscopic observations of nasal epithelium after experimental alteration of air flow. *Ann Otol Rhinol Laryngol* 79 451.
- Holopainen E 1967 Nasal mucous membrane in atrophic rhinitis with reference to symptomfree nasal mucosa. *Nord. J. Med.*
- Nous Presse Med 6 1281.
- Malm L & Wihl J Å 1976 Intranasal beclomethasone dipropionate in vasomotor rhinitis. *Acta Allergol (Kbh)* 31 245.
- Mygind N 1977 *Nasal Allergy Ultrastructure Immunology Disease Therapy*. Blackwell Oxford.
- Mygind N & Johnsen N J 1977 Significance of nasal smear eosinophilia (in preparation).
- Mygind N, Pedersen C H, Prytz S & Sørensen H 1975 Treatment of nasal polyps with intranasal beclomethasone dipropionate aerosol. *Clin Allergy* 5 159.
- Mygind N, Thomsen J & Jørgensen M B 1974a Ultrastructure of the epithelium in atrophic rhinitis. Scanning electron microscopic studies. *Acta Otolaryngol (Stockh)* 77 439.
- Mygind N, Viner A S & Jackman N 1974b Histology of nasal mucosa in normals and in patients with perennial rhinitis. *Rhinology* 12 131.
- Pedersen C B, Mygind N, Sørensen H & Prytz H 1976 Long term treatment of nasal polyps with beclomethasone dipropionate aerosol. II Clinical results. *Acta Otolaryngol (Stockh)* 82 260.
- Thiringer G, Eriksson N, Malmberg M & Svedmyr N 1975 Bronchoscopic biopsies of bronchial mucosa before and after beclomethasone dipropionate therapy. *Postgrad Med J* 51 30.
- N. Mygind M.D.  
Otorhinolaryngological Laboratory  
Rigshospitalet  
DK-2100 Copenhagen  
Denmark.

IDENTIFICATION OF  $\beta$ -ADRENOCEPTORS AND HISTAMINE RECEPTORS IN THE CAT NASAL VASCULATURE

C R Hiley, H Wilson and M S Yates

*From the Department of Pharmacology and Therapeutics University of Liverpool Liverpool England*

(Received June 4 1977)

**Abstract** The responses of the nasal capacitance vessels of the cat were recorded following intra arterial injections of  $\beta$  adrenoceptor agonists and histamine  $H_1$  and  $H_2$  receptor agonists. Isoprenaline evoked vasodilation and propranolol ( $\beta$  adrenoceptor antagonist) caused a parallel shift to the left of the dose-response curve. Metamizol ( $H_1$  antagonist) produced a further small parallel shift to the right of the histamine log dose-response curve after the administration of mepyramine ( $H_1$  antagonist). It is concluded that the nasal capacitance vessels of the cat contain  $\beta_2$ -adrenoceptors and  $H_1$  and  $H_2$  histamine receptors.

produced nasal congestion in the dog. This was not antagonized by  $H_1$ -receptor antagonists at doses used clinically. Recently, the study of histamine receptors has been greatly facilitated by the development of specific agonists and antagonists for  $H_1$ - and  $H_2$ -receptor (Black et al., 1972, Flynn & Owen, 1975) and both types of receptors have been shown to participate in the responses to histamine in a number of vascular beds (Flynn & Owen, 1975, Powell & Brody, 1976). In order to identify the types of histamine receptors present in the nasal vasculature we have studied the effects of some compounds active on both histamine  $H_1$ - and  $H_2$ -receptors.

## METHOD

Cats of either sex weighing 2-3 kg were anaesthetized with sodium pentobarbitone ( $40 \text{ mg kg}^{-1}$ ), the trachea and the cephalic vein of one fore-limb cannulated and the systemic blood pressure recorded from a femoral artery by a Bell & Howell pressure transducer (type 4-327-L221). Constrictions and dilatations of the nasal blood vessels were recorded respectively as decreases and increases in pressure in the sealed right nasal cavity using a cannula inserted into the nostril and a type 4 327 L22

Although several workers have attempted to identify  $\beta$  adrenoceptors in the nasal blood vessels, their existence remains a subject of some debate. Hall & Jackson (1968) could not demonstrate  $\beta$  adrenoceptors in the nasal vasculature of the dog, nor could Anggård & Edwall (1974) in their studies on the exchange vessels of the cat. However, in another series of experiments, evidence was obtained in the cat to suggest that  $\beta$ -receptors of the  $\beta_2$  type are present (Malm, 1974b, 1977). One of the aims of the present investigation was to re-examine this problem.

In contrast to the several studies on adrenoceptors, the histamine receptors that are also present within the nasal vasculature have received little attention despite the widespread use of histamine  $H_1$ -antagonists in allergic rhinitis. However, Bentley & Jackson (1970) showed that exogenous histamine and endogenous histamine released by compound 48/80

This work was supported by a United Kingdom Medical Research Council Project Grant to Dr H. Wilson.

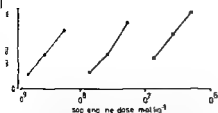


Fig. 1 Effects of propranolol on the log dose-response curve for isoprenaline acting on the capacitance vessels of the cat nasal mucosa.  $\circ$  Responses in the absence of agonist  $\bullet$  responses in the presence of  $3.4 \times 10^{-7}$  mol kg $^{-1}$  propranolol  $\square$  responses in the presence of  $3.7 \times 10^{-6}$  mol kg $^{-1}$  propranolol. Vasodilation is expressed as increases in pressure in the sealed nasal cavity (in cm H $_2$ O) and each point is the mean of three responses.

Bell & Howell pressure transducer (Wilson & Yates 1975). This method only records the overall vascular changes in the nose and will mainly reflect the changes in the capacitance vessels (Anggård & Edwall 1974; Malm 1974a). Recordings were made on a Devices M2 recorder.

Agonists were injected in 50–400  $\mu$ l isotonic saline into the right external carotid artery by means of a cannula placed in the right lingual artery. Injection of up to 600  $\mu$ l saline by this route had no effect either on the nasal vessels or on the systemic blood pressure. Antagonists were administered into the cephalic vein as a slow intravenous injection with the exception of metiamide which was given by a continuous infusion at a flow rate of 0.12 or 0.29 ml min $^{-1}$ .

The effects of antagonists are expressed in terms of the mean dose ratio  $\pm$  S.E.M., i.e. the factor by which the agonist dose must be increased in the presence of an antagonist in order to produce vasodilator responses of the same magnitude as those obtained before addition of the antagonist. Tests of significance were made by Student's paired  $t$  test.

The following drugs were used: isoprenaline hydrochloride (Pharmax), salbutamol sulphate (Allen & Hanbury), propranolol hydrochloride (ICI), phentolamine mesylate (CIBA), metipyrmine maleate (May & Baker), metiamide

2-(2-aminoethyl)pyridine and 4-methylhistamine (SK & F Laboratories).

## RESULT

### $\beta$ -adrenoceptor studies

Injections of isoprenaline in 4 cats produced dose-related vasodilation. This response of the nasal vessels to isoprenaline was antagonized by the administration of propranolol (Fig. 1). The mean dose ratio for isoprenaline produced by  $3.4 \times 10^{-7}$  mol kg $^{-1}$  propranolol was  $17.3 \pm 5.6$ . At  $3.7 \times 10^{-6}$  mol kg $^{-1}$  propranolol the isoprenaline dose ratio was  $160 \pm 8.9$ .

Nasal vascular responses to injections of salbutamol ( $7.0 \times 10^{-11}$  to  $7.0 \times 10^{-9}$  mol kg $^{-1}$ ) were compared with those produced by isoprenaline ( $1.6 \times 10^{-11}$  to  $1.0 \times 10^{-10}$  mol kg $^{-1}$ ) in 10 cats. Both drugs evoked vasodilation and the log dose-response curves were approximately parallel in all the experiments. The equipotent molar ratio of salbutamol/isoprenaline was  $8.8 \pm 1.1$ .

### Histamine receptor studies

Injections of histamine 2-(2-aminoethyl)pyridine (an  $H_1$  agonist; Flynn & Owen 1975) and 4-methylhistamine (predominantly an  $H_2$ -agonist; Flynn & Owen 1975) produced dose-dependent vasodilation in the nasal vessels. The log dose-response curves were parallel (Fig. 2) and equipotent molar ratios were determined in 11 cats. The ratio of the potency of 4-methylhistamine/histamine was  $17.2 \pm 3.1$ .

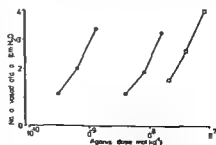


Fig. 2 Log dose-response curves for three agonists active at histamine receptors.  $\circ$  Histamine  $\bullet$  4-methylhistamine  $\square$  2-(2-aminoethyl)pyridine. Each point is mean of three determinations.



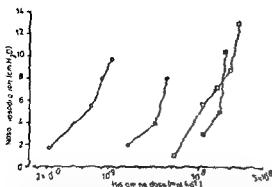


Fig 3 Log dose histamine-vasodilation curves showing the effects of increasing doses of mepyramine. ○ Control responses. ● □ and ■ responses in the presence of  $2.4 \times 10^{-7}$ ,  $2.6 \times 10^{-6}$  and  $7.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$  mepyramine respectively. Each point is the mean of three responses.

whilst that for 2-(2-aminoethyl)pyridine/histamine was  $45.0 \pm 2.9$ .

Mepyramine antagonized the effects of histamine (Fig 3) but the use of this antagonist was complicated by the intense vasoconstriction it produced in the nasal vasculature. In the experiment illustrated in Fig 3 injection of  $2.6 \times 10^{-6}$  mol  $\text{kg}^{-1}$  mepyramine produced a decrease in pressure in the sealed nasal cavity which reached a maximum of 7.4 cm H<sub>2</sub>O within 3 min and then declined slowly, disappearing after 20 min. In 2 animals it proved impossible to record responses of the nasal vessels to agonists after this dose of mepyramine because of the intense vasoconstriction it produced. Accordingly, the dose was restricted to  $1.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$  in later experiments.

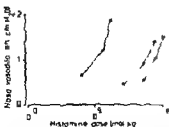


Fig 4 Effects of antagonists on log dose response curves for histamine. ○ Responses of the nasal capacitance vessels to histamine before administration of antagonists. ● responses after  $1.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$  mepyramine. □ responses in the presence of mepyramine ( $1.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$ ) and metiamide ( $2.3 \times 10^{-6}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$ ).

This dose produced a mean dose ratio of  $7.9 \pm 1.3$  in 6 cats and subsequent infusion of metiamide ( $2.3 \times 10^{-6}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$ ) gave further small (though significant,  $p < 0.05$ ) rightward shift in the histamine log dose-response curve in all the animals (mean dose ratio  $9.6 \pm 3.5$ ). One experiment is shown in Fig 4.

Mepyramine ( $1.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$ ) in 5 cats produced only a very small mean shift in the log dose-response curve to the H<sub>2</sub>-agonist methylhistamine (mean dose ratio  $1.5 \pm 0.6$ ) but subsequent infusion of metiamide ( $2.3 \times 10^{-6}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$ ) produced a parallel shift (mean dose ratio  $57.2 \pm 12.7$ ). In contrast, the response to 2-(2-pyridine after mepyramine was biphasic with a brief non dose related vasodilation followed by vasoconstriction of greater duration. This vasoconstriction could be considerably reduced or abolished by intravenous phenylamine ( $2.6 \times 10^{-6}$  mol  $\text{kg}^{-1}$ ).

Infusion of metiamide alone, at rates  $9.3 \times 10^{-7}$  and  $2.3 \times 10^{-6}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$  or had significant effects ( $p < 0.05$ ) on the log dose-response curve for 4-methylhistamine. With this agonist the shift in the curve was parallel for each infusion rate and the faster rate produced the larger shift. The mean dose ratios were  $16.4 \pm 26.9$  and  $68.4 \pm 96.8$  respectively. In the 2 cats that received mepyramine during the faster metiamide infusion there was a further rightward shift of the curve (mei-

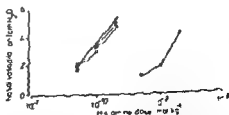


Fig 5 Effects of antagonists on the log dose-response curves for histamine. ○ Responses before administration of antagonists. ● responses after metiamide ( $9.3 \times 10^{-7}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$ ). □ responses after metiamide ( $2.3 \times 10^{-6}$  mol  $\text{kg}^{-1} \text{ min}^{-1}$ ) and mepyramine ( $1.2 \times 10^{-6}$  mol  $\text{kg}^{-1}$ ).

dose ratio 151). There was a significant shift in the log dose-response curve to histamine only when mepyramine was administered during metamide infusion (Fig. 5). The log dose-response curve to 2-(2-aminoethyl)pyridine was not significantly altered by either infusion of metamide but again became biphasic when mepyramine was given during the infusion.

## DISCUSSION

Previous pharmacological investigations of the nasal vasculature have demonstrated the presence of  $\alpha$ -adrenoceptors in this vascular bed (Hall & Jackson, 1968; Malm, 1974a) but there is disagreement concerning the presence of adrenoceptors.  $\beta$ -receptors were not detected in the capacitance vessels of the dog (Hall & Jackson, 1968) or in the exchange vessels of the cat (Anggård & Edwall, 1974). On the other hand, Malm (1974b) found evidence for the presence of  $\beta$ -receptors in the resistance vessels of the cat nasal mucosa but his results for the capacitance vessels were inconclusive.

The experiments with isoprenaline reported here clearly show that dilation of the nasal capacitance vessels can be produced by close intral injection of this  $\beta$ -agonist. Furthermore, this response is antagonized in a dose-dependent manner by propranolol, a  $\beta$ -antagonist. Dilation of the vessels was also produced by administration of salbutamol and comparison of the potencies of the two agonists gave a mean isoprenaline/salbutamol activity ratio of 8.8 which is within the range of activity ratios for tissues presumed to contain mainly  $\beta_2$ -receptors (Schild, 1973). These results show that the nasal capacitance vessels of the cat contain  $\beta$ -adrenoceptors of the  $\beta_2$ -subtype which is the subtype that Malm (1974b) concluded were present in the resistance vessels.

Further pharmacological investigation of the nasal capacitance vessels shows that both types of histamine receptor are present since  $H_1$ - and  $H_2$ -agonists produce vasodilation

which can be antagonized by the appropriate antagonists. 2-(2-aminoethyl)pyridine acts specifically at  $H_2$ -receptors and the potency ratio relative to histamine in the nasal vascular bed corresponds closely to the value of 43.7 reported for other vasculatures (Flynn & Owen, 1975). Also, whilst 4-methylhistamine is not specific to  $H_2$ -receptors, the equipotent molar ratio of 17.2 in the nasal mucosa is intermediate between that for  $H_1$ - and  $H_2$ -receptors elsewhere (200 and 2.67 respectively, Flynn & Owen, 1975).

The vasoconstrictor response to 2-(2-aminoethyl)pyridine following mepyramine treatment may be due to the release of catecholamines from the adrenal medulla as a result of the large doses employed passing through the nasal vessels into the systemic circulation. It is also possible that it represents a direct sympathomimetic action.

The antagonists also produce results closely comparable to those from other vascular beds (Flynn & Owen, 1975). Thus the  $H_2$ -receptor antagonist metamide had no effect on the response to histamine unless it was given in the presence of the  $H_1$ -antagonist mepyramine. Together the antagonists metamide and mepyramine produced greater antagonism than either one acting alone when used against histamine or 4-methylhistamine, agonists active at  $H_1$ - and  $H_2$ -receptors. Also mepyramine appeared to have a maximum effect against histamine with a dose ratio of between 16 and 19 at a dosage of  $2.6 \times 10^{-6}$  mol kg<sup>-1</sup> but the vasoconstriction produced by mepyramine restricted the experiments that could be performed. The value for the maximum dose ratio corresponds closely to those reported by Flynn & Owen (1975) for the vascular beds of the hind limb (16.3) and superior mesenteric region (13.8) of the cat consistently obtained with  $2.5 \times 10^{-5}$  mol kg<sup>-1</sup> mepyramine.

The experiments with histamine antagonists suggest that the combined use of  $H_1$ - and  $H_2$ -antagonists may be of therapeutic value in treatment of allergic rhinitis. However, it may be that histamine antagonism is not

of the effectiveness of  $H_1$ -antagonists as has been suggested by Bentley & Jackson (1970). These workers suggested that the direct vasoconstrictor effect could be the mechanism of action of these drugs and experiments in the present study have shown that strong vasoconstriction is produced at doses of mepyramine which produce only moderate blockade of histamine induced vasodilation.

In conclusion, the experiments described in this study show that the nasal capacitance vessels of the cat contain  $\beta_2$ -adrenoceptors and  $H_1$ - and  $H_2$  receptors for histamine.

### ACKNOWLEDGEMENTS

Metiamide 2 (2-aminoethyl)pyridine and 4-methylhistamine were the kind gift of Dr D. A. A. Owen of Smith Kline & French Laboratories.

### ZUSAMMENFASSUNG

Die Wirkungen von intraarteriell verabreichten  $\beta$ -Adrenorezeptoragonisten und von Histamin  $H_1$  und  $H_2$ -Rezeptoragonisten wurden auf die Kapazitätsblutgefäße der Nasenschleimhaut der Katze untersucht. Isoprenalin (loste eine Vasodilatation aus und Propranolol ( $\beta$ -Adrenorezeptor antagonist) hatte eine parallele nach rechts verschobene Log-Dosis-Wirkungskurve zur Folge. Histamin 2 (2-aminoethyl)pyridin ( $H_1$ -Agonist) und 4-methylhistamin (eigentlich  $H_2$ -Agonist) bewirkten eine Vasodilatation. Metamid ( $H_2$ -Antagonist) bewirkte eine weitere aber geringfügige parallele Verschiebung der Log-Dosis-Wirkungskurve des Histamins nach rechts nach Verabreichung von Mepyramin ( $H_1$ -Antagonist). Daraus kann man schließen, daß die Kapazitätsblutgefäße der Nasenschleimhaut der Katze  $\beta_2$ -Adrenorezeptoren sowie  $H_1$ - und  $H_2$ -Histaminrezeptoren besitzen.

### REFERENCES

- Anggård A & Edwall L 1974 The effects of sympathetic nerve stimulation on the tracer disappearance rate and local blood content in the nasal mucosa of the cat. *Acta Otolaryngol* (Stockh) 77: 131.
- Bentley A J & Jackson R T 1970 Changes in the patency of the upper nasal passage induced by histamine and antihistamines. *Laryngoscope* 80: 187.
- Black J W, Duncan W A M, Durant C J, Ganley C M & Parsons E M 1972 Definition and antagonism of histamine  $H_2$  receptors. *Nature* (Lond) 24: 385.
- Flynn S B & Owen D A A 1975 Histamine receptors in peripheral vascular beds in the cat. *Br J Pharmacol* 35: 181.
- Hall L J & Jackson R T 1968 Effects of alpha and beta adrenergic agonists on nasal blood flow. *Am J Otol Rhinol Laryngol* 77: 1120.
- Malm L 1974a Responses of resistance and capacitance vessels in the feline nasal mucosa to vasoactive agents. *Acta Otolaryngol* (Stockh) 78: 90.
- 1974b  $\beta$  adrenergic receptors in the vessels of the cat nasal mucosa. *Acta Otolaryngol* (Stockh) 78: 241.
- — — — — 1975 Histamine receptors in physiological vasodilator responses. *Am J Physiol* 231: 1002.
- Schild H O 1973 Receptor classification with special reference to  $\beta$  adrenergic receptors. In *Drug receptors: a symposium* (ed H P Rang), pp 29-36. Macmillan, London.
- Wilson H & Yates M S 1975 Crossed sympathetic innervation of the cat nasal vasculature. *J Physiol* (Lond) 247: 4P.
- H Wilson M D PhD  
Dept of Pharmacology and Therapeutics  
University of Liverpool  
P O Box 147  
Liverpool L69 3BX  
England

# RHINOLOGICAL FINDINGS FOLLOWING TRANSANTROSPHENOIDAL SURGERY OF THE PITUITARY GLAND

A Karduck and W J Bock

*From the Departments of Otorhinolaryngology and Neurosurgery  
University Hospital Essen BRD*

(Received July 27, 1977)

**Abstract** Fifty patients with pituitary gland tumours on whom we operated via the transantrosphenoidal route were rhinologically followed up with a view to revealing conceivable functional impairments incurred by this method. Our investigations included rhinoscopy, endoscopy of the nasal and paranasal cavities and of the epiglottis. X-ray controls and probes of the nasolacrimal duct and trigeminal ganglion. Apart from the technical advantages of transantrosphenoidal pituitary gland surgery, the results also confirmed its benefit to functional performance.

Three rhinological access routes to the sella turcica are predominant in the surgery of pituitary adenomas (Fig. 1)

1 The transeptal route *ad modum* Hirsch (1909, 1952), is favoured by neurosurgeons when modified according to Guiot (1969, 1976), Guiot et al (1959, 1967, 1972), Fahlbusch et al (1973, 1976), Kautzky et al (1976), Schürmann (1974), Samu & Schürmann (1976), unless the pituitary gland tumour is operated on transcranially.

2 The transethmoido-sphenoidal approach according to Chari (1912) used by a number of rhinosurgeons (Nager, 1940, Escher, 1962, 1965, 1974, Angell James, 1967, Burian, 1967, 1969, 1974, 1976).

3 The transantrosphenoidal operation method according to Denker (1921), which leads via the sinus maxillaris and the ethmoidal cell system into the sphenoidal sinuses and to the sella. Hamberger (1976) has used this rhinosurgical procedure since 1955, and from his pioneer work in pituitary surgery, he and his co-workers (1960, 1961, 1964, 1969, 1974)

gathered their experience gained on 450 patients operated on with this method.

The aim of this paper is to report our experience, with rhinological findings, following transantrosphenoidal surgery on the sella turcica.

## MATERIAL AND METHOD

Of our own 65 patients treated by transantrosphenoidal surgery of the pituitary gland (Karduck & Bock, 1977), we have followed up rhinologically 50 patients for periods of from 5 months to 3½ years, at the latest, at regular intervals (Fig. 2). Case history and ENT diagnosis ascertained for these patients prior to operation failed to reveal any pathological conditions. There were 15 cases of deviation of the septum, though this did not cause any ventilatory disturbance.

## RESULTS

In the following, the non desired side effects attributable to this operative technique are reported on.

Blood crusts seen by means of the rhinoscope in the early postoperative phase following removal of tamponade, were met with in all cases but could be quickly cleared up by means of irrigations.

Problems were caused, however, by those crusts appearing in the nasal cavity on the op-

TRANSSEPTAL ROUTE  
HIRSCH 1910

GUIOT (1957-1961)  
740 CASES

TRANSETHMOIDOSPHEOIDAL  
ROUTE (CHIARI 1912)

ANGELL JAMES (1960-1967)  
350 CASES  
BURIAN (1961-1977)  
330 CASES

TRANSANTROSPHEOIDAL  
ROUTE (DENKER 1921)

HAMBERGER (1955-1976)  
450 CASES



Fig 1 The three common rhinological routes in the sella turcica.

erated side, which formed from dried secretion and which differed distinctly from the a/m in appearance. This formation is an unmistakable sign of a severe impairment of the respiratory epithelium. It occurred in 5 patients, and we believe it to be due to a temporary local ischemia caused during operation by pressure exerted by the operating instrument, with consequent injury to the mucosa. The crustation stopped in all instances, though it necessitated treatment with unguents and salt water rinsing of the nose for months, plus frequent rhinological controls.

Another undesired effect appeared in 2 cases, viz. large adhesions of the mucosa of the lateral nasal wall to the septum causing severe impairment of nasal ventilation. Fine synechiae without disturbance of ventilation were encountered 7 times in the nasal cavity. The two adhesions had to be cut through in order to restore nasal ventilation.

When using the transantrophenoidal route the front wall of the ethmoid is opened wide,

necessitating in some instances a resection of the posterior part of the septum and of the posterior ends of the conchae; this did not lead in any case to any functional impairment.

We paid special attention to liquorrhea and meningitis which may occur postoperatively; we found them in one case each as a transient complication. However, since changing over to blocking the sella with a bone fragment taken from the facial sinus wall in addition to the usual closure of the sella with a free muscle graft, we have not seen any such complications. We found that this bony plate adapts easily during the healing process, a fact proved by X ray and endoscopic controls.

The sensibility probe of the fifth cranial nerve revealed in all cases a hypesthesia within the supply area of its second branch, though this normalized in every instance during the first postoperative weeks. No disturbances in the sense of smell occurred.

Septum perforation was not found in any of our patients.

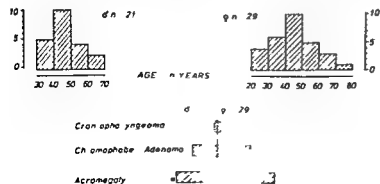


Fig 2 Age set and diagnosis in 40 cases of transantrophenoidal surgery of pituitary adenomas.

## DISCUSSION

Recently Kinnman (1973) has published in a clinical study on 80 patients treated by transantrosphenoidal surgery a report of postoperative local reactions as follows. A moderate swelling of the soft tissues corresponding to the antral opening was seen in most patients but it subsided during the course of a week to 10 days. A moderate nasal discharge was usually seen during the first 3-4 weeks. As a rule the patients also experienced some hypesthesia in the cheek and nasolabial area lasting for periods up to 3 months. In one patient an oro-antral fistula persisted postoperatively, but closed spontaneously 4 months after the operation. In one patient an indolent swelling below the area of the right lacrimal sac developed one year after the operation and the nasolacrimal duct was obstructed on the same side; an isolated ethmoidal mucocele was found. In another patient a moderate epiphora due to obstruction of the naso lacrimal duct on the side of the transantrosphenoidal operation has developed 4 years postoperatively. Hyposmia or anosmia has never been encountered nor has an atrophic rhinitis developed in any patient. Postoperative complications in form of liquorrhea and meningitis were unusual.

Fahlbusch (1977), a neurosurgeon who has used the transeptal route, found crustation and septum perforation to be the most frequent complications.

We are indebted to Bunan (1977) for the clearest statement, drawn from his ample case material and controls gathered over many years, according to this: a mucocele of the frontal sinus seems to be a very rare occurrence, though a typical complication of the transthemoido-sphenoidal treatment of pituitary gland tumours.

For our 50 transantrosphenoidally operated patients endonasal crustation is the predominant complaint. Nevertheless we must stress in agreement with the above mentioned authors (Kinnman, Fahlbusch, Bunan) that meningitis and liquorrhea which are the most

dangerous postoperative sequelae, are very rarely met with for all three rhinological operative methods.

## ZUSAMMENFASSUNG

50 Patienten mit Hypophysentumoren, die wir auf transantrosphenoidalem Weg operierten, wurden rhinologisch nachuntersucht zur Überprüfung der Frage, welche Funktionsbeeinträchtigungen als Folge dieser Methode auftreten. Die Untersuchungen umfaßten die Rhinoskopie, die Endoskopie der Nasenhaut und Nebenhöhlen sowie des Nasenrachens, Röntgenkontrollen und Nifaktorius und Nifingunus-Prüfung. Die Ergebnisse zeigten neben den operationstechnischen Vorteilen der transantrosphenoidalen Hypophysenchirurgie auch ihren funktionschonenden Charakter auf.

## REFERENCES

- Angell James J 1967 Transethmoidal hypophysectomy. *Arch Otolaryngol* 86: 256.
- Bunan K 1967 Transsphenoidal operation for tumours of the pituitary gland. *Arch Otolaryngol* 86: 449.
- 1969 Die Hypophysektomie aus der Sicht des Rhinologen. *HNO (Berlin)* 17: 193.
- 1974 Rhinochirurgische Eingriffe im Hypophysenbereich. *Arch Otorhinolaryngol* 207: 401.
- 1976 A two-stage neuro-rhinological procedure in the treatment of pituitary tumours. In *Clinical Micro-neurosurgery* (ed W Th Koos, F W Bock & R F Spetzler). Thieme Stuttgart.
- 1977 Personal communication.
- Charrat O 1912 Über eine Modifikation der Schloffer'schen Operation von Tumoren der Hypophyse. *Wiener Klin Wochenschr* 25: 5.
- Denker A 1921 Zur Behandlung der Hypophysentumoren. *Verhandlungen der Gesellschaft Dtsch Hals-Nasen- und Ohrenärzte Leipzig* 3: 134.
- Escher F 1967 Technik und Ergebnis der transthemoidosphenoidalen Hypophysektomie. *Dtsch Med J* 13: 325.
- 1965 Die paranasale transthemoido-sphenoidale Hypophysektomie. In *Advances in Oto-Rhino-Laryngology* (ed L Ruedi) vol 12, p 105. S. Karger, Basel.
- 1974 Rhinochirurgische Eingriffe im Keilbein-Hypophysenbereich. *Arch Otorhinolaryngol* 207: 409.
- Fahlbusch H 1977 Personal communication.
- Fahlbusch R & Marguth F 1973 Klinik und Behandlung der Hypophysenadenome. *Deutsches Arzteblatt für Fortbildung* 27: 1795.
- Fahlbusch R, Rjosk H K & von Werder H 1976 Operative treatment of prolactin-producing adenomas. *European Workshop on Treatment of Pituitary Adenomas*, October 28-30, 1976, Rottach-Egern, Tegernsee, Germany. Abstracts.
- Guio G 1969 L'exérèse transsphenoidale des adénomes hypophysaires (expérience de 251 interventions). *Corso superiore sui tumori delle ghiandole endocrine*. Milano, pp 109-125.

- 1976 Indications for transcranial and transsphenoidal operations. European Workshop on Treatment of Pituitary Adenomas October 28-30 1976 Rottach-Egern Tegernsee Germany Abstracts
- Guiot G, Bouche J & Opron A 1967 Les indications de l'abord transsphénoïdal des adénomes hypophysaires. Expérience de 165 interventions. *Presse Med* 75 1563
- Guiot G & Derome P 1972 Les indications de la voie d'abord transsphénoïdale en neurochirurgie. Expérience de 521 interventions. *Ann Med Interne (Paris)* 123 703
- Guiot G, Thibaut B & Bourreau M 1959 Extirpation des adénomes hypophysaires par la voie transseptale et transsphénoïdale. *Ann Otolaryngologie (Paris)* 76 1017
- Hamberger C A 1969 Transantro-sphénoïdale Hypophysenoperationen. *HNO (Berlin)* 17 317
- Hamberger C A 1976 Personal communication
- Hamberger C A & Hammer G 1964 Transsphénoïdale Hypophysektomie. In *Handbuch der Hals-Nasen-Ohrenheilkunde* (ed J Berendes, R Link & F Zollner) Bd I p 801. G Thieme Stuttgart
- Hamberger C A, Hammer G, Norlen G & Sjogren H 1960 Surgical treatment of acromegaly. *Acta Otolaryngol (Stockh)* Suppl 158 168
- 1961 Transantrosphénoïdal hypophysectomy. *Arch Otolaryngol* 74 22
- Hamberger C A & Kinnman J 1974 Transsphénoïdale Chirurgie der Hypophyse. In *Kopf und Hals Chirurgie* (ed H H Naumann) Bd 2 p 629. G Thieme Stuttgart
- Hirsch O 1909 Eine neue Methode der endonasalen Operation von Hypophysentumoren. *Wiener Med Wochenschrift* 59 636
- 1952 Symptoms and treatment of pituitary tumours. *Arch Otolaryngol* 55 268
- Karduck A & Bock W J 1978 Zur transsphénoïdalen Chirurgie von Hypophysentumoren. *Chirurgische Praxis* (in press)
- Kautzky H, Ludecke D, Lucke Ch, Nowakowski H, Schrader B, Solbach H-G, Wiegelmann W & Stahnke N 1976 Transsphénoïdale Operation in acromegaly. European Workshop on Treatment of Pituitary Adenomas October 28-30 1976 Rottach-Egern Tegernsee Germany Abstracts
- Kinnman J 1973 Acromegaly: an ultrastructural analysis of 51 adenomas and a clinical study in 80 patients treated by transantrosphénoïdale operation. Thesis pp 15 17 35 36. Norstedt & Söner Stockholm
- Nager F H 1940 The paranasal approach to intrasellar tumours. *J Laryngol Otol* 55 361
- Sami M & Schurmann A 1976 Operative treatment in relation to location and extension of pituitary adenomas. European Workshop on Treatment of Pituitary Adenomas October 28-30 1976 Rottach-Egern Tegernsee Germany Abstracts
- Schurmann A 1974 Diskussionsbemerkung zu A. Bumann. Rhinochirurgische Eingriffe im Hypophysenbereich. *Arch Otorhinolaryngol* 207 446
- Dr med A Karduck  
Universitäts Hals-Nasen-Ohrenklinik  
Hufelandstr 55  
D-4300 Essen I BRD

## ELECTROMYOGRAM OF THE TENSOR TYMPANI MUSCLE IN MAN DURING SWALLOWING

B Salen and J E Zakrisson

*From the Department of Otolaryngology University Hospital Umeå Sweden*

(Received July 5 1977)

**Abstract** Experiments were carried out on 2 patients who underwent an operation for chronic otitis media where upon the tensor tympani muscle was visualized. A unipolar platinum electrode was inserted into the muscle. Daily EMG recordings were made during swallowing and other motor activity. Distinct pronounced EMG activity was recorded from both patients every time they swallowed. It was concluded that the tensor tympani muscle participates in the act of swallowing and thereby probably contributes to the ventilation of the middle ear.

Though the structure of the middle ear muscles, the stapedius and the tensor tympani, was first described more than 400 years ago (Eustachius, 1562, Varolius, 1575) their function in man has not been studied in detail prior to the most recent decades. The function of the stapedius muscle has been elucidated in a number of investigations (e.g. Fletcher, 1962, Borg & Zakrisson, 1974, Zakrisson, 1974). In contrast the function of the tensor tympani muscle in man is much less clear. Acoustically induced tensor tympani reflexes have been reported by Terkildsen (1957, 1960), but Klockhoff (1961) considered this phenomenon to be merely a part of a startle response. Liden et al (1970) discovered acoustically induced tensor tympani reflexes in 13% of normal individuals tested. Jepsen (1955) and Zakrisson (1975) found a total absence of a tensor tympani reflex upon acoustic stimulation in patients with peripheral facial palsy including stapedius muscle paralysis.

The following methods have been used to study the activity of the tensor tympani muscle in man:

(a) recording of the changes in impedance of the ear (Jepsen, 1955, Klockhoff, 1961)

(b) measurement of changes in position of the eardrum (Terkildsen, 1957, Holst et al, 1963, Ingelstedt & Jonson, 1967, Liden et al, 1970)

(c) direct visual observation of the tensor tympani tendon (Djupestrand, 1964)

(d) electromyography (Salomon & Starr, 1963, Djupestrand, 1965)

As a rule, tactile stimulation of the regions around the eyes and ears and acoustic stimulation have been used to induce the tensor tympani reflex. Tactile stimulation of the external ear region possibly elicits tensor tympani muscle activity in man, which is interpreted by most researchers as one component in a startle response (Klockhoff, 1961, Djupestrand, 1964). A tensor tympani muscle contraction having the character of a reflex has been thought to arise even in connection with motor activity in the tensor palati muscle. EMG activity in the tensor tympani muscle in association with vocalization and swallowing has been reported by Salomon & Starr (1963) and Djupestrand (1965). Tensor tympani muscle activity has been recorded during belching and yawn by means of volume-flow measurements (Ingelstedt & Jonson, 1967).



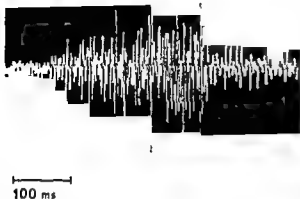


Fig. 1. EMG recording obtained during swallowing with a unipolar platinum electrode in the tensor tympani muscle belly and with a reference electrode in the ipsilateral membranous ear canal.

The tensor tympani muscle differs as to anatomical configuration in animals and man. In the cat, the muscle consists solely of pars petrosa, while in the rabbit and the guinea pig it includes both a pars petrosa and a pars tubaria, in man only the pars tubaria portion is present (Kirkhae, 1960). The tensor palati muscle is embryologically related to the tensor tympani muscle and has the same motor innervation, the trigeminal nerve (Hamilton et al., 1959).

The aim of the present investigation was to study whether the tensor tympani muscle is activated in the act of swallowing.

## MATERIAL AND METHODS

The experiments were carried out on 2 patients with chronic otitis media with cholesteatoma. Radical mastoidectomy was performed in both cases under local anesthesia. The amount of anesthetic fluid (1% Xylocaine-exadrine) was kept as low as possible. The operation revealed that both patients had cholesteatoma in the entire epitympanum and the middle ear and parts of the ossicles were absent. The attic and the middle ear were opened widely with a drill and with the aid of a microscope, cholesteatoma granulation tissue and all remaining parts of the ossicles, except the footplate of the stapes were removed. The

tendon of the tensor tympani muscle and the processus cochleariformis were identified. About 5 mm of the belly of the tensor tympani muscle was visualized by means of drilling away the processus cochleariformis and parts of the septum of the musculotubal canal.

EMG recordings from the tensor tympani muscle were obtained with a unipolar platinum electrode inserted into the muscle belly. The tip diameter of the electrode was about 10  $\mu$ m and around parts of it a glass rod had been cast. The reference electrode was a stainless steel hypodermic needle inserted into the dorsal part of the ipsilateral membranous ear canal. The experiments were carried out in an electrically shielded cage. The amplified EMG potentials were recorded on one channel of a 2-channel tape-recorder (Revov A 77) and on an ink writer (Grass Polygraph). The activity was also displayed on an oscilloscope (Tektronix 561). The patients were told to swallow repeatedly with minimal movements of the jaw- and face-muscles, to turn their heads, to yawn and move their arms. Instructions to the patients were recorded into the taperecorder's second channel. The EMG signals were analysed on a later occasion.

## RESULTS

The results from the 2 patients were similar. During rest, no EMG activity was observed. During every act of swallowing a burst of motor unit potentials was recorded from the muscle, each burst having a duration of about 300 ms. The potentials were distinct spikes clearly standing out from the background activity. See the figure, which also shows that a number of motor units within the uptake area of the electrode took part in the activity. Yawning and movements of the head gave only weak and diffuse activity, while arm movements induced no activity whatsoever.

## DISCUSSION

The results from the present study in man showed that the tensor tympani muscle con-

acted during swallowing. Swallowing is an act directed by the central nervous system and in which the ventilation of the middle ear is intimately linked by means of the opening of the Eustachian tube. The results presented here indicate that the tensor tympani muscle might play a role in the ventilation of the middle ear. Ingelstedt & Jonson (1967) observed that an average of 7.5 microlitres of gas flowed through the Eustachian tube toward the epipharynx when a tensor tympani muscle contraction took place. Borg (1972) found that transudate developed in the middle ear of rabbits in which the tensor tympani muscle had been eliminated. These observations also support the assumption that the tensor tympani muscle contributes to the ventilation of the middle ear cleft.

## ZUSAMMENFASSUNG

Die Untersuchungen wurden an zwei Patienten unter der Operation von chronischer Mittelohrentzündung durchgeführt, wobei der Musculus tensor tympani freigelegt wurde. Eine unipolare Platinelektrode wurde tief in den

...at registriert werden. Daraus ist zu schließen, daß der M. tensor tympani am Schluckakt beteiligt ist und dadurch wahrscheinlich zur Ventilation des Mittelohrs beiträgt.

## REFERENCES

- Borg E 1972 Excitability of the acoustic m. stapedius. *Acta Otolaryngol* (Stockh) Suppl 188 287
- Borg E 1972 Excitability of the acoustic m. stapedius. *Acta Otolaryngol* (Stockh) Suppl 188 287
- Djupestrand G 1964 Middle ear muscle reflexes elicited by acoustic and nonacoustic stimulation. *Acta Otolaryngol* (Stockh) Suppl 188 287
- 1965 Electromyography of the tympanic muscles in man. *Int Audiol* 4 34
- Eustachius ■ 1562 Epistola de auditus organis
- Fletcher J L 1962 Reflex response of middle ear muscles. Protection of the ear from noise. *Sound* 1 17
- Hamilton W J, Boyd J D & Mossman H W 1959 *Human Embryology*. Hefner Cambridge
- Holst H E, Ingelstedt ■ & Örtengren U 1963 Ear drum movements following stimulation of the middle ear muscles. *Acta Otolaryngol* (Stockh) Suppl 189 73
- Ingelstedt S & Jonson B 1967 Mechanisms of the gas exchange in the normal ear. *Acta Otolaryngol* (Stockh) Suppl 224 452
- Jepsen O 1955 *Studies on the Acoustic Stapedius Reflex in Man*. Thesis Universitetsforlaget Aarhus
- Kurikae I 1960 *The Structure and Function of the Middle Ear*. The University of Tokyo Press Tokyo
- Klockhoff I 1961 Middle ear muscle reflexes in man. *Acta Otolaryngol* (Stockh) Suppl 164
- Liden G, Peterson J L & Harford E R 1970 Simultaneous recording of changes in relative impedance and air pressure during acoustic and non acoustic elicitation of the middle ear reflexes. *Acta Otolaryngol* (Stockh) Suppl 263 208
- Salomon G & Starr A 1963 Electromyography of middle ear muscles in man during motor activities. *Acta Neurol Scand* 39 161
- Terkildsen K 1957 Movements of the ear drum following intra aural muscle reflexes. *Arch Otolaryngol* 66 484
- 1960 Acoustic reflexes of the human musculus tensor tympani. *Acta Otolaryngol* (Stockh) Suppl 158 230
- Varolius C 1575 *Anatomiae sive de resolutione corporis humani libri IIIII*. Francofurti: cited by Wever & Lawrence (1944)
- Zaknsson J E 1974 Experimental studies on the function of the stapedius muscle in man. Thesis Umeå University Medical Dissertations No. III
- 1975 The role of the stapedius reflex in poststimulatory auditory fatigue. *Acta Otolaryngol* (Stockh) 79 1

B. Salen M.D.  
Dept of Otolaryngol  
University Hospital  
S 90185 Umeå  
Sweden

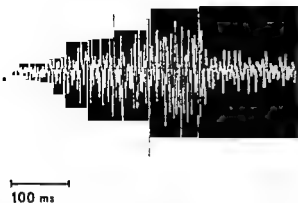


Fig. 1. EMG recording obtained during swallowing with a unipolar platinum electrode in the tensor tympani muscle belly and with a reference electrode in the ipsilateral membranous ear canal.

The tensor tympani muscle differs as to anatomical configuration in animals and man. In the cat, the muscle consists solely of pars petrosa, while in the rabbit and the guinea pig it includes both a pars petrosa and a pars tubaria, in man only the pars tubaria portion is present (Kinkade, 1960). The tensor palati muscle is embryologically related to the tensor tympani muscle and has the same motor innervation, the trigeminal nerve (Hamilton et al., 1959).

The aim of the present investigation was to study whether the tensor tympani muscle is activated in the act of swallowing.

## MATERIAL AND METHODS

The experiments were carried out on 2 patients with chronic otitis media with cholesteatoma. Radical mastoidectomy was performed in both cases under local anesthesia. The amount of anesthetic fluid (1% Xylocaine exadrine) was kept as low as possible. The operation revealed that both patients had cholesteatoma in the entire epitympanum and the middle ear and parts of the ossicles were absent. The attic and the middle ear were opened widely with a drill and with the aid of a microscope, cholesteatoma, granulation tissue and all remaining parts of the ossicles except the footplate of the stapes were removed. The

tendon of the tensor tympani muscle and the processus cochleariformis were identified. About 5 mm of the belly of the tensor tympani muscle was visualized by means of drilling away the processus cochleariformis and parts of the septum of the musculotubal canal.

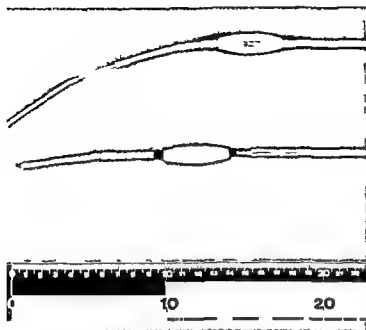
EMG recordings from the tensor tympani muscle were obtained with a unipolar platinum electrode inserted into the muscle belly. The tip diameter of the electrode was about 10  $\mu$ m and around parts of it a glass rod had been cast. The reference electrode was a stainless steel hypodermic needle inserted into the dorsal part of the ipsilateral membranous ear canal. The experiments were carried out in an electrically shielded cage. The amplified EMG potentials were recorded on one channel of a 2-channel tape-recorder (Revox A 77) and on an ink writer (Grass Polygraph). The activity was also displayed on an oscilloscope (Tektronix 561). The patients were told to swallow repeatedly with minimal movements of the jaw- and face-muscles, to turn their heads, to yawn and move their arms. Instructions to the patients were recorded into the taperecorder's second channel. The EMG signals were analysed on a later occasion.

## RESULTS

The results from the 2 patients were similar. During rest, no EMG activity was observed. During every act of swallowing, a burst of motor unit potentials was recorded from the muscle, each burst having a duration of about 300 ms. The potentials were distinct spikes clearly standing out from the background activity. See the figure, which also shows that a number of motor units within the uptake area of the electrode took part in the activity. Yawning and movements of the head gave only weak and diffuse activity, while arm movements induced no activity whatsoever.

## DISCUSSION

The results from the present study in man showed that the tensor tympani muscle con-



*Fig 1 (Upper) The guide wire with the soft flexible spring in the distal end and the bougie itself leading over the guide (Lower) The different parts of the bougie. A complete unit consists of 13 dilators from F 21 to F 45*



**Fig 2** The Eder-Puestow technique of dilating oesophageal strictures using TV fluoroscopy (SAAB Multiplane) (A) Oesophagoscopy with introduction of the guide wire

(B) The guide wire after the oesophagoscope has been removed (C) The bougie is fitted over the wire (D) the bougie is introduced into the oesophagus



Fig. 3 The guide wire in the stomach and the bougie itself in the oesophagus

### OWN SERIES

dilation system used in this trial was described by Puestow (1955) and its use in connection with the flexible oesophagoscope by Lilly & McCaffery (1971), Bennett (1972), Price et al. (1974) and Royston et al. (1976).

The dilation is performed over a guide wire of steel on the tip of which is fitted a very flexible soft spring (Fig. 1). The wire has an external diameter of 2 mm and can be inserted through the biopsy channel in the flexible oesophagoscope.

The bougie consists of a flexible tip, an olive shaped dilator in French 21 to 45 and a flexible staff of 65 cm in length.

All the patients in this trial were treated under general anesthesia. The procedure is shown in Fig. 2. Using a flexible end viewing oesophagoscope (Olympus EF) or gastroscope (Olympus GIF K), the guide wire is passed through the stricture under visual as well as TV fluoroscopy control (Fig. 3). When the spring of the wire has reached the stomach

Table I Oesophageal strictures treated with the Eder-Puestow apparatus

37 patients	108 dilations
5 patients with malignant tumour	5 dilations
32 patients with benign strictures	103 treatments

Table II Aetiology of oesophageal strictures treated with the Eder-Puestow apparatus

	N. of patients
Malignant tumour	5
Benign stricture	32
Esophageal corrosion	1
Incompetent sphincter (LFS)	25
Scleroderma	4
Miscellaneous	2



Fig 4 Incorrect technique leading to perforation of the esophagus with the Eder-Puestow dilator

Fig 4 Incorrect technique leading to perforation of the esophagus with the Eder-Puestow dilator

the scope is withdrawn, and bougies of suitable diameters are led down over the guide wire without risk of perforation.

This procedure has now been used in Copenhagen University Medical Centre, Division of Thoracic Surgery, Rigshospitalet over a 2 year period (1974-76). Thirty seven patients underwent treatment (Table I).

The etiology of stricture development (Table II) was incompetent lower oesophageal sphincter in 25 cases, scleroderma in 4 cases, oesophageal corrosion in one case, miscellaneous reasons in 2 cases and malignant tumour in 5 cases.

In these 37 patients a total of 108 dilations were performed with intervals of between 4 days and 6 months (average 2 months). No complications were seen due to this Eder-Puestow procedure.

## DISCUSSION

Once decision is made to apply bouginage to a patient with stricture of the oesophagus the Eder-Puestow dilation instrument is the instrument of choice. The risk attending conventional dilation through a stiff oesophagoscope is perforation with a mortality rate at

the same level of mortality for oesophageal resection, when performed at centres using this kind of operation.

The dilation treatment with the flexible oesophagoscope can be practicable with a safety hitherto unknown. When performed with a pliable and gentle hand, we believe that the procedure is the safest currently known and the risk of perforation hardly greater than with oesophagoscopy with fiberoptic itself, i.e. about 0.05% (Schiller et al., 1972).

Should the staff and bougie push the wire forward instead of sliding over it, the wire will no longer serve as a guide, and perforation can result (Fig 4). It is therefore essential to keep the wire taut and to ensure that the staff is sliding over it.

## ZUSAMMENFASSUNG

Die größte Sicherheit in der Bougiebehandlung enger Ösophagusstrukturen wird erzielt durch die Verwendung eines flexiblen Ösophagoskops, wodurch eine Leitsonde am Puestow eingelegt wird. Hierüber wird die Bougie ohne Gefahr von Perforation eingeführt. 108 Dilationen wurden ohne Verwicklungen vorgenommen.

## REFERENCES

- Bennett J R 1972 A safer method of dilating benign oesophageal strictures *Gut* 13 1026
- Bill Jr A H, Mebus W K & Sauvage L R 1963 Evaluation of techniques of esophageal dilation in relation to the danger of perforation *J Thorac Cardiovasc Surg* 45 510
- Borgeskov S, Egedorf J & Kruse Blinksberg H 1976 Progress in oesophagoscopy *Acta Otolaryngol (Stockh)* 87 151
- Chung M S K, Safae Shirazi M & Denbesten L 1976 Dilation of esophageal strictures *Arch Surg* 111 795
- Jackson C & Jackson C L 1955 *Bronchoesophagology* W B Saunders Co Philadelphia
- Lilly J O & McCaffery T D 1971 Esophageal stricture dilation *Am J Dig Dis* 16 1137
- Mullen D C, Sealy W C & Young W G Jr 1968 Results of twenty years experience with esophageal replacement for benign disorders *Ann Thorac Surg* 5 481
- Price J D, Stanciu C & Bennett J 1974 A safer method of dilating oesophageal strictures *Lancet* i 1141
- Puestow K L 1955 Conservative treatment of stenosing diseases of the esophagus *Postgrad Med* 18 6

- Raptis S & Milne D M 1972 A review of the management of 100 cases of benign stricture of the oesophagus *Thorax* 27 599
- Royston C M S, Dowling B L & Gear M W L 1976 Esophageal dilatation using the Eder Puestow dilators *Am J Surg* 131 697
- Schiller H, F R, Cotton P B & Salmon P R 1972 The hazards of digestive fibre endoscopy *Gut* 13 1027
- Vejlsted H & Struve-Christensen F 1977 The effect of dilatation in the treatment of benign oesophageal strictures *Scand J Throat Cardiac Surg* 11 71
- S. Borgeskov M D  
Dept of Surgery A  
Rigshospitalet  
DK 2100 Copenhagen  
Denmark

# AUTHOR INDEX

Aantaa E and Virolainen E The Pre and Postoperative ENG Findings in Clinical Otosclerosis and the Late Hearing Results	313
Alberti P W Morgan P P and Czuba I Speech and Pure Tone Audiometry as a Screen for Exaggerated Hearing Loss in Industrial Hearing Claims	328
Albrektsson T See Tjellstrom A Lindstrom J Albrektsson T Brånemark P I and Hallén O	
Albrektsson T See Tjellstrom A Lindstrom J Albrektsson T Brånemark P I and Hallén O	
Allum J H J See Koenig E Allum J H J and Dichgans J	
Anniko M and Sarkady L The Effects of Mercurial Poisoning on the Vestibular System	96
Anniko M and Sarkady L Cochlear Pathology Following Exposure to Mercury	213
Anniko M Reversible and Irreversible Changes of the Stria Vascularis	349
Arlinger S D Kylen P and Hellqvist H Skull Distortion of Bone Conducted Signals	318
Ask P and Tibbling L A Simple Device Measuring Differences in Level in the Oesophagus	296
Aust R Backlund L Drettner B Falck B and Jung B Comparative Measurements of the Mucosal Blood Flow in the Human Maxillary Sinus by Plethysmography and by Xenon	111
Axelsson A and Vertes D Vascular Histology of the Guinea Pig Cochlea	198
Axelsson A and Lindgren F Hearing in Pop Musicians	225
Bak Pedersen K See Parving A and Bak Pedersen K	
Beagley H A See Legoux J P Teas D Beagley H A and Remond M C	
Benitez J T See Bouchard K R and Benitez J T	
Bock W J See Karduck A and Bock W J	
Boedts D and Kuypers W Epithelial Migration on the Tympanic Membrane	248
Booth J H See Moffat D A Gibson W P H Ramsden R T Morrison A W and Booth J B	
Borg E Peripheral Vasoconstriction in the Rat in Response to Sound I Dependence on Stimulus Duration	153
Borg E Peripheral Vasoconstriction in the Rat in Response to Sound II Dependence on Rate of Change of Sound Level	332
Borgeskov S and Struve Christensen E The Modern Treatment of Oesophageal Strictures Using the Eder Puestow Dilators	456
Bouchard K R and Benitez J T Ultrasonic Irradiation through the Round Window	372
de Brey H B and Eggermont J J The Influence of Cochlear Temperature on the Electrical Travelling Wave Pattern in the Guinea Pig Cochlea	363
Brånemark P I See Tjellstrom A Lindstrom J Albrektsson T Brånemark P I and Hallén O	
Backlund L See Aust R Backlund L Drettner B Falck B and Jung B	
Carenfelt C and Lundberg C The Role of Local Gas Composition in Pathogenesis of Maxillary Sinus Empyema	116
Carlsoo B and Östberg Y Ultrastructural Observations on the Parotitis Autoimmunica in the NZB/NZW Hybrid Mice	298
Casselbrant M Ingelstedt S and Ivarsson A Volume Displacement of the Tympanic Membrane at Stapedius Reflex Activity in Different Postures	1
Ceballos A See Ciges M Gonzalez M and Ceballos A	
Ciges M Gonzalez M and Ceballos A Desquamation on Taste Buds	



- Cramer D B Graybiel A and Oosterveld W J Successful Transfer of Adaptation  
Acquired in a Slow Rotation Room to Motion Environments in Navy Flight Training 74
- Czuba I See Alberti P W Morgan P P and Czuba I
- Dahl H A See Teig E Dahl H A and Thorkelsen H
- Dichgans J See Koenig E Allum J H J and Dichgans J
- Drettner B See Aust R Backlund L Drettner B Falck B and Jung B
- Eccles R The Domestic Pig as an Experimental Animal for Studies on the Nasal  
Cycle 431
- Eggermont J J See de Brey H H and Eggermont J J
- Enksson H See Hiraide F and Enksson H
- Firbas W See Wicke W Welleschik B Firbas W and Sinzinger H
- Falck B See Aust R Backlund L Drettner B Falck B and Jung B
- Fredrickson J M See Rubin A M Liedgren S C R Ödkvist L M Milne  
A C and Fredrickson J M
- Gibson W P R See Moffat D A Gibson W P R Ramsden R T Morrison  
A W and Booth J B
- Gjulling E V and Melnikov O F Cytolytic Activity of Tonsil Cells 149
- Gonzalez M See Ciges M Gonzalez M and Ceballos A
- Graybiel A See Cramer D B Graybiel A and Oosterveld W J
- Gundersen T and Molvaer O I Hearing Loss Resulting from Perilymph Fistula 374
- Hallen O See Tjellström A Lindström J Albrektsson T Bränemark P I and  
Hallén O
- Hallen O See Tjellström A Lindström J Albrektsson T Bränemark P I and  
Hallén O
- Hellqvist H See Arlinger S D Kylen P and Hellqvist H
- Hiley C W Wilson H and Yates M S Identification of  $\beta$  Adrenoceptors and  
Histamine Receptors in the Cat Nasal Vasculature 444
- Hiraide F and Enksson H The Effects of the Vacuum on Vascular Permeability  
of the Middle Ear 10
- Hoffman H S See Marsh R R Hoffman H S and Stitt C
- Homick J L See Igarashi M Takahashi M and Homick J L
- Igarashi M Takahashi M and Homick J L Optokinetic Afternystagmus and  
Postrotatory Nystagmus in Squirrel Monkeys 387
- Igarashi M See Takahashi M Igarashi M and Reschke M F
- Iijima M See Taguchi K Iijima M and Suzuki T
- Ingelstedt S See Casselbrant M Ingelstedt S and Ivarsson A
- Ishida M Die Lactatdehydrogenase (LDH) des Innenohres nach Larmbelastung 17
- Ivarsson A See Casselbrant M Ingelstedt S and Ivarsson A
- Jung B See Aust R Backlund L Drettner B Falck B and Jung B
- Kambic V See Radsel Z and Kambic V
- Kinnikunen A See Rosenhall U Nylén O Lindberg J and Kinnikunen A
- Karduck A and Bock W J Rhinological Findings Following Transantrosphenoidal  
Surgery of the Pituitary Gland 449
- Kawabata I and Nomura Y Extra Internal Hair cells 342
- Koenig E Allum J H J and Dichgans J Visual Vestibular Interaction upon  
Nystagmus Slow Phase Velocity in Man 397
- Kortekangas A I Nordman E M and Voutilainen A Experience with Preopera-  
tive Irradiation in Head and Neck Cancer 122
- Kringlebotn M See Molvaer O I Vallersnes F M and Kringlebotn M
- Kuypers W See Boedts D and Kuypers W
- Kylén P See Arlinger S D Kylen P and Hellqvist H

Lechner Steinheitner S	See Schone H and Lechner Steinheitner S	
Legoux J P	Teas D C Beagley H A and Remond M C: Relation between the Waveform of the Cochlear Whole Nerve Action Potential and its Intensity Function	177
Lindberg J	See Rosenhall U Nylen O Lindberg J and Kankkunen A	
Lindgren F	See Axelsson A and Lindgren F	
Lindstrom J	See Tjellstrom A Lindström J Albrektsson T Bränemark P I and Hallen O	
Lombardi R	See Stefanelli M Mira E Schmid R and Lombardi R	
Lundberg C	See Carenfelt C and Lundberg C	
Lundquist P G	Schindler R A and Stahle J Ultrasonic Irradiation of the Guinea Pig Labyrinth	85
Manley G A	Cochlear Frequency Sharpening—A New Synthesis	167
Marsh R R	Hoffman H S and Stitt C Reflex Inhibition Audiometry	336
Melnikov O F	See Gjuling E V and Melnikov O F	
Melville Jones G and Young L R	Subjective Detection of Vertical Acceleration A Velocity Dependent Response	45
Milne A C	See Rubin A M Liedgren S C H Ödkvist L M Milne A C and Fredrickson J M	
Mira E	See Stefanelli M Mira E Schmid R and Lombardi R	
Moffat D A	Gibson W P R Ramsden R T Morrison A W and Booth J B Trans tympanic Electrocochleography during Glycerol Dehydration	158
Molvaer O I	Vallersnes F M and Kringlebotn M The Size of the Middle Ear and the Mastoid Air Cell	24
Molvaer O I	See Gundersen T and Molvaer O I	
Morgan P P	See Alberti P W Morgan P P and Czuba I	
Morrison A W	See Moffat D A Gibson W P R Ramsden R T Morrison A W and Booth J B	
Mygind N	Sørensen H and Pedersen C B The Nasal Mucosa during Long Term Treatment with Beclomethasone Dipropionate Aerosol	437
Nathanson A	The Early Vascularization of an Autogenous Bone Inlay into an Artificial Defect in the Rabbit Mandibula	63
Nomura Y	See Kawabata I and Nomura Y	
Nordman E M	See Kortekangas A E Nordman E M and Voutilainen A	
Nylen O	See Rosenhall U Nylen O Lindberg J and Kankkunen A	
Oosterveld W J	See Gerner D R Gushki A J O	
Pallestrini E A	See	
Palva T	Thesleff I Cholesteatoma Epithelium	307
Parving A	Reliability of Bekesy Threshold Tracing in Identification of Carriers of Genes for an X Linked Disease with Deafness	40
Parving A and Bak Pedersen K	Clinical Findings and Diagnostic Problems in Sensorial Neural Low Frequency Hearing Loss	184
Pedersen C H	See Mygind N Sørensen H and Pedersen C H	
Petrosini L	See Troiani D Petrosini L and Pallestrini E A	
Pirodda E and Rinaldi Ceroni A	Some Experiments on Temporary Thresholds Shifts Produced by Short Tones	191
Radsel Z and Kambic V	The Influence of Cigarette Smoke on the Pharyngeal Mucosa	128
Ramsden R T	See Moffat D A Gibson W P R Ramsden R T Morrison A W and Booth J B	
Remond M C	See Legoux J P Teas H C Beagley H A and Remond M C	

- Reschke M F See Takahashi M Igarashi M and Reschke M F
- Rinaldi Ceroni A See Pirodda E and Rinaldi Ceroni A
- Rosenhall U Nylen O Lindberg J and Kanlkunen A Auditory Function after Haemophilus Influenzae Meningitis 243
- Rubin A M Liedgren S C R Ödkvist L M Milne A C and Fredrickson J M Labyrinthine and Somatosensory Convergence upon Vestibulo-Ocular Units 64
- Salén B and Zakrisson J E Electromyogram of the Tensor Tympani Muscle in Man during Swallowing 453
- Sarkady L See Anniko M and Sarkady L
- Saxén L See Palva T Thesleff I and Saxén L
- Schindler R A See Lundquist P G Schindler R A and Stahle J
- Schmid R See Stefanello M Mira E Schmid R and Lombardi R
- Schone H and Lechner Steinleitner S The Effect of Preceding Tilt on the Perceived Vertical 68
- Sinzinger H See Wicke W Welleschik B Firbas W and Sinzinger H
- Stahle J See Lundquist P G Schindler R A and Stahle J
- Stefanello M Mira E Schmid R and Lombardi R Quantification of Vestibular Compensation in Unilateral Meniere's Disease 411
- Stutt C See Marsh R R Hoffman H S and Stutt C
- Struve Christensen E See Borgeskov S and Struve Christensen E
- Suzuki T See Taguchi K Iijima M and Suzuki T
- Sorensen H See Mygind N Sorensen H and Pedersen C H
- Taguchi K Iijima M and Suzuki T Computer Calculation of Movement of Body's Center of Gravity 420
- Takahashi M Igarashi M and Reschke M F Directional Conflict between Vestibular and Visual Inputs in the Squirrel Monkey 253
- Takahashi M See Igarashi M Takahashi M and Homick J L
- Teas D C See Legoux J P Teas D C Beagley H A and Remond M C
- Tegner H Quantitation of Human Granulocyte Protease Inhibitors in Non-purulent Bronchial Lavage Fluids 782
- Teig E Dahl H A and Thorkelsen H Actomyosin ATPase Activity of Human Laryngeal Muscles 272
- Thesleff I See Palva T Thesleff I and Saxén L
- Thorkelsen H See Teig E Dahl H A and Thorkelsen H
- Tibbling L See Ask P and Tibbling L
- Tjellström A Lindström J Albrektsson T Brånemark P I and Hallén O A Clinical Pilot Study on Preformed Autologous Ossicles Part I 33
- Tjellström A Lindström J Albrektsson T Brånemark P I and Hallén O A Clinical Pilot Study on Preformed Autologous Ossicles Part II 232
- Tokunaga O Inhibitory and Promoting Effects of Subliminal Pendular Rotation on Optokinetic Nystagmus in Man 63
- Troiani D Petrosini L and Palestini E A Neural Discharge of Medial Geniculate Body Units and Single Semicircular Canal Stimulation 267
- Vallersnes F M See Molvaer O I Vallersnes F M and Kringlebotn M
- Welleschik B See Wicke W Welleschik B Firbas W and Sinzinger H
- Vertes D See Axelsson A and Vertes D
- Wicke W Welleschik B Firbas W and Sinzinger H Zur Streptomyces-schädigung des Ganglion Spirale 360
- Wilson H and Yates M S Sympathetic Nerves and Nasal Secretion in the Cat 426
- Wilson H See Hiley C R Wilson H and Yates M S
- Virolainen E See Aantaa E and Virolainen E

- Yates M S See Wilson H and Yates M S  
 Yates M S See Hiley C R Wilson H and Yates M S  
 Young L R See Melvill Jones, G and Young L R  
 Zakrisson J E See Salén B and Zakrisson J E  
 Ödkvist L M See Rubin A M, Liedgren S C R, Ödkvist L M Milne A C  
 and Fredrickson J M  
 Östberg Y See Carlsoo B and Östberg Y

## SUBJECT INDEX

- Autogenous Bone Inlay the Early Vascularization into an Artificial Defect in the Rabbit Mandibula 63  
 Bone Conducted Signals Skull Distortion of 318  
 Cat Nasal Vasculature Identification of  $\beta$  Adrenoceptors and Histamine Receptors 444  
 Cholesteatoma Epithelium Organ Culture Studies on Human Skin 307  
 Cigarette Smoke The Influence on the Pharyngeal Mucosa 128  
 Cochlear Frequency Sharpening—a New Synthesis 167  
 Cochlear Pathology Following Exposure to Mercury 213  
 Cochlear Whole Nerve Action Potential Relation between the Waveform and its Intensity Function 177  
 Computer Calculation of Movement of Body's Center and Gravity 420  
 Electrocochleography during Glycerol Dehydration 158  
 Electromyogram of the Tensor Tympani Muscle in Man during Swallowing 453  
 Extra Internal Hair Cells 347  
 Genes for an X linked Disease with Deafness Reliability of Bekesy Threshold Tracing in Identification of Carriers 40  
 Guinea Pig Cochlea Vascular Histology 198  
 Guinea Pig Cochlea The Influence of Cochlear Temperature on the Electrical Traveling Wave Pattern 363  
 Haemophilus Influenzae Meningitis Auditory Function after 243  
 Hearing Loss in Industrial Hearing Claims Speech and Pure Tone Audiometry as a Screen 328  
 Human Laryngeal Muscles Actomyosin ATPase Activity 272  
 Human Maxillary Sinus by Plethysmography and by Xenon Comparative Measurements of the Mucosal Blood Flow 111  
 Innenohre nach Lärmbelastung die Lactatdehydrogenase (LDH) 17  
 Mastoid Air Cell The Size of the Middle Ear 24  
 Maxillary Sinus Empyema The Role of Local Gas Composition in the Pathogenesis 116  
 Middle Ear The Effects of the Vacuum on Vascular Permeability 10  
 Nasal Cycle The Domestic Pig as an Experimental Animal for Studies 431  
 Nasal Mucosa during Long Term Treatment with Beclomethasone Dipropionate Aerosol 437  
 Nasal Secretion in the Cat and Sympathetic Nerves 426  
 Non Purulent Bronchial Lavage Mucuses Quantitation of Human Granulocyte Protease Inhibitors 282  
 Nystagmus Slow Phase Velocity in Man Visual Vestibular Interaction 397  
 Oesophagus A Simple Device Measuring Differences in Level -  
 Oesophageal Structures The Modern Treatment Using the Eder Puestow Dilators  
 Optokinetic Afternystagmus and Postrotatory Nystagmus

Optokinetic Nystagmus in Man Inhibitory and Promoting Effects of Subluminal Pendular Rotation	63
Parotitis Autoimmunica Ultrastructural Observations in the NZB/NZW Hybrid Mice	298
Perilymph Fistula Hearing Loss Resulting from	324
Pop Musicians Hearing in	225
Pre and Postoperative ENG Findings in Clinical Otosclerosis and the Late Hearing Results	313
Preceding Tilt the Effect on the Perceived Vertical	68
Preformed Autologous Ossicles A Clinical Pilot Study Part I	33
Preformed Autologous Ossicles A Clinical Pilot Study Part II	232
Preoperative Irradiation Experience in Head and Neck Cancer	122
Reflex Inhibition Audiometry	336
Response to Sound Peripheral Vasoconstriction in the Rat	
I Dependence on Stimulus Duration	153
II Dependence on Rate of Change of Sound Level	332
Round Window Ultrasonic Irradiation	372
Semicircular Canal Stimulation Neural Discharge of Medial Geniculate Body Units and Single	262
Sensori Neural Low Frequency Hearing Loss Clinical Findings and Diagnostic Problems	184
Slow Rotation Room Successful Transfer of Adaptation Acquired to Motion Environments in Navy Flight Training	74
Stapedius Reflex Activity Volume Displacement of the Tympanic Membrane in Different Postures	1
Streptomycinschädigung des Ganglion Spirale	360
Stria Vascularis Reversible and Irreversible Changes	349
Taste Buds Desquamation on	290
Temporary Threshold Shifts Produced by Short Tones Some experiments	191
Tonsil Cells Cytolytic Activity	149
Transantrosphenoidal Surgery of the Pituitary Gland Rhinological Findings	449
Tympanic Membrane Epithelial Migration	248
Ultrasonic Irradiation of the Guinea Pig Labyrinth	85
Unilateral Meniere's Disease Quantification of Vestibular Compensation	411
Vestibular and Visual Inputs in the Squirrel Monkey Directional Conflict	253
Vestibular System The Effects of Mercurial Poisoning	96
Vestibulo-Ocular Units Labyrinthine and Somatosensory Convergence	54
Vertical Acceleration Subjective Detection A Velocity Dependent Response	45

# *Acta* OTO-LARYNGOLOGICA

VOL. 85 • MAY-JUNE 1978 • No. 5-6

EDITOR: C-A. HAMBERGER • STOCKHOLM

## EDITORIAL BOARD

DENMARK: O. ELBROND • O. JEPSEN

H. K. KRISTENSEN • N. RISKÆR • H. SØRENSEN • P. STOKSTED

FINLAND: J. KÄRJÄ • O. H. MEURMAN • A. PALVA • T. PALVA

NORWAY: J. HALL • E. STEEN • P. WINTHER

SWEDEN: G. ASCHAN • B. BARR • H. DIAMANT • B. DRETTNER • C. H. ENGSTRÖM

H. ENGSTRÖM • O. HALLÉN • S. INGELSTEDT • J. WERSÄLL

DISTRIBUTED BY  
THE ALMQVIST & WIKSELL PERIODICAL COMPANY  
STOCKHOLM, SWEDEN

## COLLABORATORS

- Austria* L. Hörbst, F. Krejci, E. H. Mayer, O. Novotny, E. Schlönder, S. Unterberger  
*Canada* D. P. Bryce, J. Fredrickson, W. J. McNally, J. A. Sullivan  
*Denmark* J. Falbe Hansen, Th. Vilstrup  
*Finland* H. Björk, B. Grahne, U. Surala, E. Vaheri  
*France* M. Aubry, L. G. Chevance, G. Greiner, P. L. Mounier Kuhn, M. Portmann  
*Germany* A. Herrmann, H. G. Loebell, A. Michlke, R. Mittermaier, H. H. Naumann, K. H. Vosteen, H. Wullstein, F. Zöllner  
*Great Britain* G. H. Bateman, I. S. Hall, D. F. N. Harrison, R. D. Owen  
*Greece* J. Chryssikos, L. Papangelou, G. E. Yannoulis  
*India* J. V. De Sa, A. B. N. Rao, C. Satyanarayana, P. N. Sinha  
*Italy* M. Arslan, E. Bocca, F. Brunetti  
*Japan* T. Dalto, T. Fukuda, M. Goto, I. Kinkae, M. Morimoto, J. Ono, S. Sato  
*Netherlands* L. B. W. Jongkees, W. H. Struben  
*Norway* P. Berdal, H. F. Fabritius, T. Leegaard, O. Ophcim, S. Quist Hansen, O. Strömme  
*Sweden* G. Dohlman, G. Herberts, L. Holmgren, H. Koch, G. Lidén, N. Lundgren, C. O. Nylén, A. Sjöberg  
*Switzerland* F. Escher, E. Lüscher, A. Montandon, C. R. Pfaltz, L. Rüedi, J. P. Taillens, A. Weder  
*USA* L. F. Boies, J. E. Bordley, T. Cody, D. A. Hilding, P. H. Holinger, H. P. House, G. Kelemen, F. L. Lederer, J. R. Lindsay, H. F. Schuknecht, B. H. Sentuna, G. E. Shambaugh, Jr., F. A. Sooy, W. P. Work  
*USSR* M. Kchodiakov, S. Khechinashvili, N. A. Preobrazhensky

## *Conferences and Meetings*

1978 June 1-3 Ordinary meeting of the Bárány Society to be held at the Dept. of Otolaryngology, Akademiska sjukhuset, S 750 14 Uppsala, Sweden.

1978 June 1-3 Mayo Clinic Course 'Current Medical and Physiological Aspects of Rhinology' Addr. Clifford F. Lake, Mayo Clinic, Rochester, MA 55901

1978, June 5-16 A two-week Temporal Bone Surgical Dissection Course will be held at the Ear Research Institute in Los Angeles Addr. Antonio De La Cruz, 256 South Lake Street, Los Angeles CA 90057

1978 June 19-23 A Postgraduate Course with a Seminar on hypopharyngeal carcinoma and cervical esophagus will be held in Milano Italy Addr. Miss Lucia Manfredi, Istituto Nazionale Tumori, Via G. Venezian 1, 20133 Milano Italy

1979 July 22-27 The Fifth British Academic Conference in Otolaryngology will be held at the University of Birmingham UK Addr. V. Hammond FRCS., 55 Harley Street, London W1

